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***In vivo* triglyceride composition of abdominal adipose tissue measured by ¹H MRS at 3T**

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Abstract

Purpose—To investigate the regional variability of adipose tissue triglyceride composition *in vivo* using ¹H MRS, examining potential confounders and corrections for artifacts, to allow for adipose tissue spectrum estimation.

Materials and Methods—¹H MR STEAM spectra were acquired *in vivo* at 3 Tesla from 340 adult patients (mean age 48.9, years range 21–79 years; 172 males, 168 females; mean body mass index [BMI] 34.0, range 22–49 kg/m²) with known or suspected NAFLD in deep (dSCAT), surface (sSCAT) subcutaneous adipose tissue, and visceral adipose tissue (VAT). Triglyceride composition was characterized by number of double bonds (ndb) and number of methylene-interrupted double bonds (nmidb). A subset of patients (dSCAT n = 80, sSCAT n = 55, VAT n = 194) had the acquisition repeated three times to examine the repeatability of ndb and nmidb estimation.

Results—Mean ndb and nmidb showed significant ($p < 0.0001$) differences between depots except for dSCAT and sSCAT nmidb (dSCAT ndb 2.797, nmidb 0.745; sSCAT ndb 2.826, nmidb 0.737; VAT ndb 2.723, nmidb 0.687). All ndb and nmidb estimates were highly repeatable (VAT ndb ICC = 0.888, nmidb ICC = 0.853; sSCAT: ndb ICC = 0.974, nmidb ICC = 0.964; dSCAT: ndb ICC = 0.959, nmidb ICC = 0.948).

Conclusion—Adipose tissue composition can be estimated repeatably using ¹H MRS and different fat depots have different triglyceride compositions.

Keywords

Magnetic Resonance Spectroscopy; Adipose Tissue; Triglyceride Composition; Fatty Acids

Introduction

The development of imaging techniques to measure adipose tissue depot volumes has allowed the relationship between depot volume and metabolic disease to be well characterized (1,2). Abdominal superficial subcutaneous adipose tissue (sSCAT), deep subcutaneous adipose tissue (dSCAT), and visceral adipose tissue (VAT) have distinct morphological, metabolic, and genetic characteristics (3,4). While it has been suggested that each depot also has a distinct triglyceride composition, only one small scale study has used MRS to examine differences between dSCAT and sSCAT (5) and this distinction has not yet been characterized in all 3 depots in a large scale study of adults. *Ex vivo* studies comparing fat depot composition have been restricted to small scale studies in patients undergoing surgery (6-9). In-vivo studies have used ^{13}C MRS, but this method requires specialist hardware, long acquisition times, and large acquisition volumes, limiting its ability to study individual depots (10,11).

Proton magnetic resonance spectroscopy (^1H MRS) offers the possibility of determining the triglyceride composition of adipose tissue *in vivo* at clinical field strengths (3 Tesla) (1,12,13). Each of the resonance peaks present in the fat ^1H MR spectrum (Figure 1) represents a distinct proton moiety (Table 1) (14,15). Spectroscopy can specify the chemical structure of the triglyceride in terms of three variables: number of $-\text{CH}=\text{CH}-$ double bonds per molecule (ndb), number of double bonds separated by a single CH_2 (nmdb - number of methylene-interrupted double bonds), and the fatty acid chain length (CL) (16). As ^1H MRS is non-invasive and can be performed in relatively small voxels with reasonably short acquisition times, it allows acquisition of spectra from multiple locations in the adipose tissue (1,17,18).

Characterization of the adipose fat spectrum may also improve the accuracy of multi-echo, gradient-echo MRI techniques that incorporate the spectral structure of triglyceride to give fat and water images (19,20). Using an adipose spectrum in these techniques may allow more accurate fat-water imaging than that provided those incorporating spectral models derived from food oils or hepatic triglycerides (21).

The purpose of this study is to investigate adipose tissue triglyceride composition *in vivo* using ^1H MRS. Potential confounders and corrections for artifacts were investigated *in vivo* and using phantoms. The characterization of each abdominal adipose depot allows the fat spectra of the individual adipose tissue depots and mean adipose triglyceride spectrum to be determined.

Materials and Methods

The clinical component of this project was a prospective, single-site study approved by our Institutional Review Board and compliant with the Health Insurance Portability and

Accountability Act. Three-hundred-and-forty adult patients were recruited from clinical studies being conducted at our institution and provided informed written consent. These patients either had nonalcoholic fatty liver disease (NAFLD), were at risk of NAFLD, were obese, or had lipodystrophy and were undergoing research exams investigating NAFLD. The adipose MRS sequences were additional to the main studies. Patients were enrolled and underwent research MR exams between January 2010 and December 2014.

MRS Acquisition

All *in vivo* ^1H MRS spectra were acquired at 3 Tesla (GE Signa EXCITE HD, GE Healthcare, Waukesha, WI) using an 8-channel torso array coil. The Stimulated Echo Acquisition Mode (STEAM) sequence was chosen to allow the shortest minimum TE of 10 ms, and the mixing time (TM) for the STEAM sequence was fixed at a minimum value of 5 ms. The TM and TE were chosen to minimize J coupling effects (17,22,23). A TR of 3,500 ms was chosen to minimize T1 effects. There was no water saturation, and spatial saturation bands around the voxel were disabled to ensure a uniform spectral response across the frequency range of interest.

Main MRS Acquisition

After conventional imaging, a $15 \times 15 \times 15$ mm voxel on the right side of the body was selected in the following locations (Figure 2): 1) dSCAT; 2) sSCAT; and 3) retro-peritoneal VAT (1,2). The location of the voxel was primarily constrained by body habitus. The retro-peritoneal VAT generally allowed only one voxel location. The dSCAT voxel was generally selected at the L1-L2 vertebrata level, with the sSCAT voxel selected on same slice if possible. If required, the voxel size dimensions were altered slightly to fit the fat depot without reducing the total volume of the voxel by more than a third.

All spectra were shimmed during free breathing. Spectra were acquired in SCAT with 16 signal averages and two pre-acquisition excitations during free breathing, giving a one minute acquisition. However the VAT spectra, being the more susceptible to breathing motion, were collected in a 25 s breath-hold acquisition with six signal averages and a single pre-acquisition excitation.

All patients underwent the main MRS acquisition. Subsets of patients also underwent additional MRS acquisitions in addition to the main MRS acquisitions. The first eight patients also had T2 estimation. From then onwards, the decision to acquire additional MRS acquisitions depended on convenience of the schedule and patient comfort constraints; the data acquired in this study was collected on patients undergoing long exams and scanner availability and patient tolerance of more scans decided if extra acquisitions were attempted.

Sub Acquisition 1 - T2 Estimation

In the first subset of 8 patients, spectra were acquired in the dSCAT using a long-TR, multi-TE STEAM sequence to allow estimation of the T2 of the fat peaks (16). Five single average STEAM spectra (TM 5 ms) were acquired consecutively at progressively longer TEs of 10, 15, 20, 25 and 30 ms in a single acquisition. The TE range and TM was chosen to minimize J coupling effects (23). A TR of 3,500 ms was chosen to minimize T1 effects. This allowed

the measurement of T2 of the individual fat peaks, as the different fat peaks have different T2s (17). The measured T2 were then use to correct for T2 of the TE 10 ms spectra.

Sub Acquisition 2 – Repeatability

To examine the repeatability of adipose characterization in each depot, a second subset of patients had the dSCAT, sSCAT and VAT acquisition repeated two more times with identical parameters to examine the repeatability of ndb and nmidb estimates for dSCAT, sSCAT and VAT. All spectra were acquired without any repositioning of the patient. For this sub-acquisition, 80 patients had three spectra acquired from dSCAT, 55 patients had three spectra acquired from sSCAT and 194 patients had three spectra acquired from VAT.

Sub Acquisition 3 – Left vs. Right Variability

In an unpublished pilot study, we incidentally observed that the estimates of adipose tissue fat composition in the left and right side of the body differed. Assuming there is no intrinsic left-right biological bias, any difference must be due to positional dependence of the response of the scanner. To confirm this observation and to develop analysis techniques to minimize this positional dependence, a third subset of 18 patients had MRS performed in the deep subcutaneous adipose tissue on the left side of the body in addition to the dSCAT, sSCAT, and VAT measurements made on the right. The left dSCAT and right dSCAT acquisition parameters were identical, and the left dSCAT voxel was positioned to mirror as much as possible the location of the right dSCAT location (Figure 2). The positional dependence response of the scanner was also investigated with phantoms (discussed below.)

Phantom Study of Artifactual Region Variability

As well as examining the positional dependence response of the scanner *in vivo*, the possibility of artifactual regional variability was examined in an olive oil phantom scanned using an identical sequence to that used in dSCAT. A bottle phantom (diameter 7 cm) was placed at a height above the scanner table typical of the dSCAT voxel location. The phantom was scanned at 21 evenly-spaced positions from 200 mm left of isocenter to 200 mm right of isocenter.

MRS Analysis

The spectra from the individual channels were combined using a singular value decomposition based approach (24). A single experienced observer analyzed the spectra using the AMARES algorithm (25) (included in the MRUI software package) (26). All the fat peaks were modeled by multiple Gaussian resonances. The 1.3 ppm peak was modeled by four Gaussians; the fat peaks at 5.3, 4.2, 2.1, and 0.9 ppm were each modeled by two Gaussians, while the 2.7 ppm peak was modeled by a single Gaussian. The water peak was also modeled by a single Gaussian. While the peaks were loosely constrained to avoid non-physical solutions, the amplitude, linewidth or frequency of the peaks were otherwise fitted freely. In the subset of patients with spectra taken at multiple TEs, the T2-values of the spectral peaks and the T2-corrected peak areas were calculated by non-linear least-square fitting. These T2 values were used to correct all the single TE spectra acquired in this study. Spectra were judged of inadequate quality to measure ndb and nmidb, if it was impossible to

clearly distinguish the individual fat peak from each other, or a large water peak indicated significant signals from non-adipose tissues. Patients were excluded from the analysis if acceptable spectra from two voxel locations were not acquired.

A detailed description of the MRS method employed to calculate ndb and $nmdb$ has already been published (12). Briefly, the relative area of each peak was found by adding the number of hydrogen nuclei with its associated type of bond in the triglyceride molecule. This gives the relative area of the peaks shown in Table 1. As allowing the chain length (CL) to vary freely produced unstable ndb and $nmdb$ estimates, CL was fixed at 17.5 to match that measured in adipose tissue by Ren et al (17). The ndb and $nmdb$ values were calculated by non-linearly minimizing the difference between the measured areas of the fat peaks and that given by the theoretical model (Table 1). Peak 2 was not included in the calculation because J coupling effects were non-negligible even at the shortest possible TE (23).

Previous studies have used a set of peaks over a limited portion of the spectrum, rather than over the full fat spectral range to minimize errors from non-uniform excitation profiles (17,18). To examine this possibility, the ndb and $nmdb$ values were calculated for two different cases: the five-peak model which used peaks 1, 3, 4, 5 and 6 to estimate saturation, and the four-peak model which used only peaks 3,4,5 and 6 to investigate if using a reduced ppm range gave different results. An identical analysis was completed for the olive oil phantom except the CL was fixed at 17.74 (27).

To determine the mean fat spectrum from each depot, the areas of the peaks were calculated from the ndb and $nmdb$ values, rather than direct measurement. This allows the area of the strongly J-coupled peak 2 to be calculated as well as allowing the fat spectrum to be calculated from an analysis which does not use the full fat spectrum, such as the four-peak model and that used in Ren et al (17). A mean adipose tissue spectra was also calculated from a non-weighted average of the three depots to provide a fat spectrum for abdomen fat-water imaging where all three depots are present.

Ren et al (17) reports triglyceride composition assuming that adipose consists of saturated, monounsaturated, and di-unsaturated triglyceride. In this case, the di-unsaturated fraction = $nmdb/3$, the monounsaturated fraction = $(ndb - 2*nmdb)/3$, and the saturated fraction = $1 - (ndb - nmdb)/3$. This allows triglyceride composition given by monounsaturated, and di-unsaturated fractions to be compared with triglyceride composition measured by ndb and $nmdb$.

Statistical Analysis

Study sample characteristics were summarized. Mean T2 in the subset of patients who had the long-TR, multi-TE STEAM acquisition was calculated and used for T2 correction in all patients. Paired t-tests were used to assess differences in ndb and $nmdb$ in dSCAT, sSCAT and VAT, and between right and left dSCAT. Intraclass correlation coefficient (ICC) was used to estimate the repeatability of the ndb and $nmdb$ as estimated in the dSCAT, sSCAT and VAT. A two-tailed significance level $p < 0.05$ was considered statistically significant.

Results

Patient Demographics are shown in Table 2. Of the 340 patients, 24 were excluded from the analysis because they did not have usable spectra for two distinct adipose locations. A total of 316 patients had spectra acquired in at least two locations, with all 316 having spectra from dSCAT, 224/316 from sSCAT and 301/316 from VAT. The most common reason for a depot not having usable spectra was the there was insufficient volume of adipose tissue for a spectral voxel to be selected in that depot. In these cases, no acquisition was attempted or the spectra from that depot displayed signal contamination from surrounding tissue.

Table 1 summarizes the T2 values of each of the fat peaks as estimated by the long-TR, multi-TE sequence in a subset of eight patients. T2 values of the CH₂ peak at 4.2 ppm could not be accurately estimated due to J coupling.

Table 3 compares mean ndb and nmdb values for right and left dSCAT calculated using the five- and four-peak models. There are no significant differences between left and right for the four-peak model but significant differences are seen in the five-peak model. The table also compares for ndb and nmdb, the correlation between right and left dSCAT for the five- and four-peak models, and there is higher correlation seen comparing left and right values in the four-peak model than there is in the five-peak model. This shows the reduced ppm range of the four-peak model increases the agreement between left and right values, compared to the five-peak model.

The variation in ndb and nmdb in the olive oil phantom with position is displayed in Figure 3. The five-peak model shows a positional dependence in ndb and nmdb with both values increasing as the voxel moves from left to right. There is less positional dependence in the four-peak model. The four-peak and five-peak model show improved agreement close to isocenter. Given the lower positional dependence of the four-peak model, only results from the four-peak model will be given henceforth.

The mean and range of ndb and nmdb of the VAT, sSCAT and dSCAT are shown in Figure 4 with the mean ndb, nmdb and spectrum for each depot summarized in Table 4. An average fat spectrum of the three depots which may represent the best compromise for MR fat-water imaging the abdomen is also given. For each fat depot, the relationship between nmdb and ndb was similar, with ndb and nmdb strongly correlated (VAT: nmdb = 0.64, ndb = 1.06, $r = 0.88$; sSCAT: nmdb = 0.60, ndb = 0.98, $r = 0.80$; dSCAT: nmdb = 0.67, ndb = 1.12, $r = 0.87$). Table 4 also compares the fat spectra with those from previous estimates of fat spectra produced using similar short-echo STEAM methodology.

Paired t-test comparison of triglyceride composition in the three depots and pairwise correlations of triglyceride composition of the three fat depots are shown in Table 5. The adipose tissue depots have significantly different values of ndb, but there was no difference between dSCAT and sSCAT for nmdb. Weaker correlations are seen between fat depots than are seen when comparing ndb and nmdb in a single fat depot. For both ndb and nmdb, the strongest correlation is seen between the VAT and dSCAT, and the weakest correlation is seen between VAT and sSCAT.

All ndb and nmdb estimates in all three depots were highly repeatable. The repeatability seen in VAT (ndb ICC = 0.888, 95% CI 0.860 - 0.911; nmdb ICC = 0.853, 95% CI 0.818 - 0.883) was slightly lower than that seen in the other depots (sSCAT ndb ICC = 0.974, 95% CI 0.960 - 0.984; sSCAT nmdb ICC = 0.964, 95% CI 0.945 - 0.978; dSCAT ndb ICC = 0.959, 95% CI 0.942 - 0.972; dSCAT nmdb ICC = 0.948, 95% CI 0.926 - 0.965).

Discussion

This study examined the feasibility of using ^1H MRS at 3T to measure the type of triglyceride present in three different fat depots. This study showed that the triglyceride composition could be measured reliably, and that different triglyceride compositions could be detected in the fat depots examined.

Differences in the triglyceride composition of the three depots suggest they have dissimilar metabolic activities, in agreement with previous volume studies which found that obesity-related diseases show stronger correlation to the volume of some fat depots and weaker correlation with others (1-4).

The ndb and nmdb values between fat depots are moderately to poorly correlated, in keeping with their dissimilar metabolic activity. Particularly when comparing VAT to dSCAT, a high level value of ndb or nmdb in one depot does not mean the other depot will also have high ndb or nmdb. This stresses the importance of examining more than one fat depot.

In addition to expanding the clinical discrimination between adipose depots, characterization of the adipose fat spectrum may also improve the accuracy of MR imaging techniques that acquire multi-echo, gradient-echo images with an analysis that incorporates the multi-peak spectral structure of triglyceride to give fat and water images (19,28). These techniques often use spectra derived from food oils or from hepatic triglycerides whilst analyzing adipose tissue (21). Using a spectral model other than that derived from adipose tissue may introduce errors in assessing the fat fraction. By accurately determining the adipose fat spectrum, this study may allow more accurate fat-water MR imaging in adipose tissue.

Comparing the triglyceride composition estimated in this study with that reported by Ren et al (17), the two adipose tissue spectra are similar, with the Ren SCAT spectrum being identical to our dSCAT spectrum. Ren et al (17) did not differentiate dSCAT from sSCAT, but this suggests the calf spectrum he observed was in dSCAT. Bone marrow is similar but shows differences greater than those seen in the three adipose tissue depots examined in this study. The liver spectrum is different from the other spectra. This is supported by gas chromatography studies which have shown liver and adipose fat depots to have different triglyceride composition (6,29). This suggests that if the prime purpose of the MRI imaging technique is to image whole body rather than liver fat, the spectra detailed here may provide more accurate spectral fitting.

One previous study has used MRS to examine the differences in dSCAT and sSCAT (5). That study agreed with data from this study finding significant differences in ndb between the two depots, but no significant difference between nmdb, but they found different values

from those reported here and those of Ren et al (17). The data was acquired using PRESS at long TE, which may introduce confounders into the measurement of triglyceride composition due to T2 variability and J-coupling.

In the five-peak model, left dSCAT ndb and nmdb values are higher than those in right dSCAT, and this bias is also seen in the phantom. However, spatial variability of the spectral response may vary from magnet to magnet, and it is possible that the five-peak model would not demonstrate positional variability in other systems. In that case the five-peak model may be the better choice as the extra info from peak 1 may allow better estimation of triglyceride content. If regional variability of triglyceride is being examined, the possibility of positional bias must be checked. If positional bias is found, the reduced spectral range of the four-peak model will be inherently less affected by positional bias. The olive oil phantom indicates that the non-uniformity decreases as the voxel moves closer to isocenter. Agreement of the four- and five-peak models near isocenter suggests that the five-peak model is preferred close to isocenter.

The T2 used to correct for relaxation effects in the fat peaks was collected in a small subset of patients (n=8). More accurate estimates of T2 have been published since the start of the study (30). However at the start of the study when these values were obtained there were limited fat T2 values. However as the TE used to acquire the data (10 ms) is much shorter than fat T2, extreme accuracy in fat T2 is not required.

MR spectroscopy struggles in patients with low volumes of adipose tissue. While it was possible to place a voxel in all patients in dSCAT, many patients had fat distributions that precluded collection of spectrum in sSCAT or VAT. In patients with small amounts of VAT, the acquisition was sensitive to slightly different breath-holds, often requiring repeat acquisitions to collect an uncontaminated spectrum.

MR spectroscopy is technically demanding, requires analysis by experienced personnel and can only measure triglyceride in a single location. By comparison, MR imaging is easier to perform, and allows triglyceride composition to be investigated across an imaging volume. Multi-echo, gradient-echo MRI techniques are being investigated for triglyceride composition analysis (31-34). These studies have mostly been in phantoms with only limited in vivo study, but offer the possibility of development of techniques which could be used beyond specialist research centers.

The technique detailed in this study cannot quantify $\omega 3$ fatty acid. There have been elegant ^1H MRS techniques proposed that attempt detection of $\omega 3$ fatty acid, although these techniques by focusing on $\omega 3$ cannot provide ndb and nmdb estimates (35,36). *In vivo* ^1H MR spectroscopy does not provide the detailed triglyceride composition given by ex-vivo techniques such as gas chromatography or by ^{13}C NMR as it cannot estimate the abundance of individual fatty acid chains. However, it can act as a guide for what regions may be biochemically active in conditions under examination, informing the choice of location for future studies using these methodologies.

A limitation of this study was that all exams were performed on a single 3T scanner from one manufacturer. As such we cannot confirm the observed positional response bias

observed in the five-peak model is an issue beyond the specific scanner used in this study. This study did not collect biopsies from patients and hence cannot confirm that the value measured by ^1H MRS is the actual triglyceride composition, though this technique has previously been verified in phantoms (15). Future validation studies should be performed at multiple sites and on multiple vendor platforms with biopsy standards.

In conclusion, in this study we have shown that the adipose tissue composition can be estimated repeatably using ^1H MRS independent of positional bias using the reduced spectral range of the four-peak model. We have shown that different fat depots have different triglyceride composition and that the fat spectrum observed in adipose tissue is different from that previously seen in liver, and it may be better suited as the fat spectrum used in MRI methods that incorporate the multi-peak spectral structure of triglyceride if the prime region of interest is adipose tissue rather than the liver.

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Figure 1. Typical STEAM TE 10 ms free-breathing acquisition in the deep subcutaneous adipose tissue. The peak assignments of peaks 1 to 6 are given in Table 1.

Figure 2.
Typical Locations of the adipose spectroscopy voxels: 1 - sSCAT, 2 - dSCAT, 3 - VAT and 4 - left dSCAT.

Figure 3.

Comparison in an olive oil phantom of the ndb (top) and $nmidb$ (bottom) measured using the four and five-peak model, as a function of voxel position. The four-peak model demonstrated a more uniform spatial response than the five-peak model.

Figure 4.
Comparison of distribution of *ndb* (left) and *nmldb* (right) in the three adipose tissue depots.

Table 1

Relative magnitude and mean T2 in a subset of eight patients of the triglyceride peaks, from Hamilton et al (16)

Peak	Location	Assignment	Relative Magnitude	In vivo ppm	T2 (ms)
1	5.29 ppm	-CH=CH-	ndb*2	5.3 ppm	54
	5.19 ppm	-CH-O-CO-	1		
2	4.2 ppm	-CH ₂ -O-CO-	4	4.2 ppm	*
3	2.75 ppm	-CH=CH-CH ₂ -CH=CH-	nmidb*2	2.75 ppm	60
4	2.24 ppm	-CO-CH ₂ -CH ₂ -	6	2.1 ppm	54
	2.02 ppm	-CH ₂ -CH=CH-CH ₂ -	(ndb-nmidb)*4		
5	1.6 ppm	-CO-CH ₂ -CH ₂ -	6	1.3 ppm	70
	1.3 ppm	-(CH ₂) _n -	[(CL-4)*6]-(ndb*8)+(nmidb*2)		
6	0.90 ppm	-(CH ₂) _n -CH ₃	9	0.9 ppm	82

* - Peak 2 (4.2 ppm) cannot be estimated due to J coupling.

Table 2

Patient Characteristics (range where applicable)

Characteristic	Value
Patient Sex	
Male	172/340
Female	168/340
Mean age	48.9 (21 - 79)
Mean body mass index kg/m ²	34.0 (20 - 49)
Demographics	
American Indian	7
Asian	36
Black/African American	4
Native Hawaiian/Pacific Islander	3
White	164
Unreported	120
Non-Hispanic	280/340
Hispanic	60/340

Table 3

Mean values (and standard deviation) of ndb and nmidb in right and left dSCAT and correlation between the values as estimated using the four- and five-peaks models.

	right dSCAT	left dSCAT	p	Correlation
Five-peak model ndb	2.77 (0.12)	2.91 (0.19)	0.0033	0.484
Four-peak model ndb	2.75 (0.14)	2.79 (0.19)	0.26	0.651
Five-peak model nmidb	0.72 (0.10)	0.85 (0.09)	<0.0001	0.487
Four-peak model nmidb	0.70 (0.09)	0.73 (0.09)	0.18	0.635

Triglyceride composition (with standard deviation) and spectra for the three adipose tissue depots and estimates previously published using similar methodology.

Table 4

Peak	Location	Assignment	VAT	sSCAT	dSCAT	Mean	Ren (17) SCAT	Ren (17) Marrow	Hamilton (16) Liver
-	-	-	17.50	17.50	17.50	17.50	17.50	17.33	17.45
-	-	ndb	2.723 (0.191)	2.826 (0.198)	2.797 (0.173)	2.782	2.80	2.86	1.92
-	-	nmdb	0.687 (0.142)	0.737 (0.148)	0.745 (0.132)	0.723	0.75	0.74	0.32
1	5.29 ppm	-CH=CH-	5.4%	5.6%	5.5%	5.5%	5.5%	5.7%	3.7%
	5.19 ppm	-CH-O-CO-	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
2	4.2 ppm	-CH ₂ -O-CO-	3.9%	3.9%	3.9%	3.9%	3.9%	4.0%	3.9%
3	2.75 ppm	-CH=CH-CH ₂ -CH=CH-	1.4%	1.5%	1.5%	1.4%	1.5%	1.5%	0.6%
	2.24 ppm	-CO-CH ₂ -CH ₂ -	5.9%	5.9%	5.9%	5.9%	5.9%	6.0%	5.8%
4	2.02 ppm	-CH ₂ -CH=CH-CH ₂ -	8.0%	8.2%	8.1%	8.1%	8.1%	8.5%	6.2%
	1.6 ppm	-CO-CH ₂ -CH ₂ -	5.9%	5.9%	5.9%	5.9%	5.9%	6.0%	5.8%
5	1.3 ppm	-(CH ₂) _n -	59.6%	59.1%	59.3%	59.4%	59.3%	58.4%	64.2%
6	0.90 ppm	-(CH ₂) _n -CH ₃	8.9%	8.9%	8.9%	8.9%	8.9%	9.0%	8.8%

Table 5

Statistical comparisons of triglyceride composition of the three fat depots.

	VAT v sSCAT	VAT v dSCAT	sSCAT v dSCAT
n	209	301	224
ndb	r = 0.695	r = 0.271	r = 0.532
	p < 0.0001	p < 0.0001	p < 0.0001
nmdb	r = 0.710	r = 0.415	r = 0.653
	p < 0.0001	p < 0.0001	p = 0.88