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Bone marrow adiposity alterations in type 2 diabetes are sex-specific and associated with serum lipid levels

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Abstract

Type 2 diabetes (T2D) has negative effects on skeletal health. A proposed mechanism of diabetic bone disease connects hyperlipidaemia to increased bone marrow adiposity and decreased bone quality. Previous research on T1D reported positive associations between serum lipid levels and marrow adiposity, but no data exist for T2D. In addition, marrow adiposity is sex-dependent in healthy populations, but sex has not been addressed adequately in previous reports of marrow adiposity in T2D. The purpose of this study was to quantify associations of marrow adiposity and composition with T2D status, serum lipid levels, and sex.

T2D patients and normoglycemic controls (n=39/37) were included. Single voxel MR spectroscopy (MRS) was performed at the spine and tibia. Quantitative MRS outcomes of marrow adiposity and composition were calculated. Linear regression models were used to compare MRS outcomes among groups and to evaluate associations of MRS outcomes with serum lipid levels. All analyses were performed on sex-stratified sub-groups.

Total, unsaturated, and saturated fat content at the spine were lower in T2D participants compared to controls in age-adjusted models; these differences were significant in men but not in women. In our study cohort, total cholesterol, LDL, and HDL were lower in T2D participants compared to controls. Adjustment for LDL, HDL, and statin use attenuated the association of T2D status with

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unsaturated fat but not saturated fat in men. Further analysis confirmed significant associations between serum lipid levels and MRS outcomes. Specifically, we found a positive association between low-density lipoprotein (LDL) cholesterol and total marrow fat in the male T2D group, and a negative association between HDL and total marrow fat in the female T2D group.

In conclusion, our results suggest that marrow adiposity and composition are associated with lipid levels as well as T2D status, and these relationships are sex-specific.

Keywords

Type 2 diabetes; bone marrow adiposity; serum lipids; MR spectroscopy; spine

Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder and a major public health concern. While in past decades research has focused on the cardiovascular, renal, and retinal complications of T2D, more recent studies have identified clinically significant negative skeletal effects of T2D[1,2]. Patients with T2D experience 40 – 70% higher fracture incidence compared to non-diabetic people at various skeletal sites, particularly at the lower extremities[3–5]. However, bone mineral density (BMD), widely used in the assessment of bone quality and fracture risk, is normal or even elevated in T2D patients. This demonstrates that traditional quantitative methods of bone assessment fail to evaluate fracture risk accurately in the setting of T2D. Further, these traditional methods of bone assessment cannot provide insight into the biological mechanisms driving the increased fracture risk in T2D.

An inverse relationship between marrow adiposity and bone quality is increasingly recognized across a spectrum of conditions. In aging and obesity, increased marrow adiposity is correlated with osteoporosis and increased fracture risk[6–9]. Increased marrow adiposity coupled with decreased bone quality has also been documented in caloric restriction and anorexia[10]. The sparse literature describing marrow adiposity in T2D, however, is inconsistent. Sheu et al found increased spine marrow adiposity in men with diabetes compared to men without diabetes[11]. In a smaller study including both men and women with severe obesity, Yu et al similarly found increased spine marrow adiposity in those with diabetes[12]. In contrast, three studies of women found no significant differences in spine marrow adiposity between participants with diabetes and those without[13–15]. In the single publication reporting marrow adiposity at peripheral sites in T2D, Kim et al report slightly lower marrow adiposity at the tibia in women with T2D compared to controls [15].

MR spectroscopy (MRS) is a non-invasive, in-vivo technique that is widely used to quantify marrow adiposity and can additionally be used to assess the *composition* of marrow adipose tissue (BMAT), including levels of lipid saturation[16]. Saturated and unsaturated marrow lipid levels have distinct relationships with bone density and fracture outcomes (16) and therefore are important to measure in addition to total marrow adiposity. [16]. As for total marrow adiposity, however, the literature describing BMAT composition in T2D is sparse and inconsistent. However, there are indications that BMAT composition is altered in T2D,

specifically that the levels of lipid saturation are higher[13] and levels of lipid unsaturation are lower[12,14]. These findings are important and possibly clinically significant because they can be indicative of the type of marrow adipose tissue present: regulated BMAT (rBMAT) or constitutive BMAT (cBMAT). With different lipid saturation profiles and possibly different functions[17–20], they can also potentially explain increased fracture risk.

While previous research suggests that both bone quality and marrow adiposity are altered in T2D, the mechanisms are still unclear. A proposed mechanism of increased marrow adiposity and decreased bone mass is through hyperlipidemia, which promotes marrow adipogenesis through the activation of PPARγ[21]. Preferential differentiation from common precursors towards adipocyte lineage may reduce osteoblastogenesis, thereby decreasing bone quality. Although no data exist on the relationship between serum lipid levels and marrow adiposity in humans with T2D, Slade et al have reported a positive association between serum lipid levels and marrow adiposity in humans with T1D[22]. This correlation has also been documented in people without diabetes[23]. Evaluating the relationship between hyperlipidemia and marrow adiposity in T2D may reveal biological mechanisms driving bone and marrow changes in T2D, which result in increased bone fragility.

Previous publications presented contradicting data regarding the association of bone marrow adiposity with T2D, but none reported sex-stratified analyses. This may be an important knowledge gap because it has been established that marrow adiposity is sex-dependent in healthy populations[24,25]. Specifically, Griffith et al found that marrow adiposity is 10% higher in healthy females compared to males, controlling for BMD, in participants over 65 years old. These results underscore the importance of sex-stratified analyses in the evaluation of BMAT alterations in T2D.

The purpose of this study was to quantify and compare bone marrow adiposity and composition in older male and female T2D patients compared to normoglycemic controls at the spine and distal tibia. Relationships between serum lipids and bone marrow adiposity and composition markers were also evaluated. We hypothesized that (i) T2D patients would have increased bone marrow adiposity compared to controls, (ii) these relationships would be sex-specific and (iii) there would be strong positive associations between serum lipid levels and marrow adiposity in T2D.

Methods

Study participants

Men and postmenopausal women aged 50–70 years with and without T2D were recruited from UCSF and community health clinics. Participants with T2D had been diagnosed at least 3 years prior. Diagnosis was defined by the American Diabetes Association: either receipt of pharmacological treatment for 3 or more years, or HbA1c of ≥ 6.5 for 3 or more years in the case of no pharmacological treatment (diet control). Normoglycemic control participants were frequency matched on age (5-year groups), sex, and BMI categories (normal, overweight and obese).

All potential participants were screened by dual-energy X-Ray absorptiometry (DXA) and only those with a DXA T-score between 0 and −2.5 were included. In addition, health and medication history information of all potential participants was collected using a questionnaire. Potential participants satisfying any of the following criteria were excluded: 1) history of metabolic bone disease other than osteoporosis; 2) chronic gastrointestinal disease, renal, or hepatic impairment (known cirrhosis or if transaminase levels 3-fold above normal limit); 3) use of medications known to impact bone and mineral metabolism, including use of a bisphosphonate or teriparatide in the last year or for >12 months ever, current calcitonin, prednisone > 5mg daily or the equivalent glucocorticoid for > 10 days in the last 3 months, current thiazolidinedione (TZD), or thyroid hormone replacement with current thyroid stimulating hormone <0.1 mIU/L; 4) perimenopausal status; and 5) contraindications to x-ray or MR imaging. The study protocol was approved by the UCSF Committee on Human Research, and each participant provided written consent before enrollment.

Laboratory Analyses

Blood was drawn after an overnight fast and the following blood tests were performed at a CLIA certified clinical laboratory: serum glucose, creatinine, hemoglobin A1c (HbA1c), calcium, 25-hydroxyvitamin D, triglycerides, total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol.

Data Acquisition

DXA scans were performed on a single DXA scanner (Horizon A; Hologic, Marlborough, MA, USA). Areal bone mineral density (aBMD) values were acquired at the spine (L1-L4) and proximal femur (neck and total femur region). T-scores were calculated based on NHANES gender-matched reference data. MRI scans of the spine and distal tibia were performed on a 3T whole-body scanner (MR750w, GE Healthcare, Waukesha, Wisconsin, USA) using an in-table spine coil and a sixteen-channel flex coil (In-Vivo Corporation, Gainesville, FL) with auto channel selection. Before performing MRS, a sagittal T2-weighted fast spin echo (FSE) sequence (TR/TE = 4500/68ms, bandwidth = ±31.25 kHz/pixel; flip angle = 142° [vertebral] or 111° [distal tibia]; FOV = 24mm [vertebral] or 16mm [distal tibia], slice thickness = 5mm) was performed for visual inspection of bone fractures and lesions as well as for prescription of the MRS acquisition box. Single voxel MRS data were acquired using Stimulated Echo Acquisition Mode (STEAM) method with the sequence parameters: TR/TE = 3000/20ms (vertebral) or 3000/30ms (distal tibia); BW=5 kHz; data point=4096. For vertebral scans, a volume of interest (VOI: 15 × 12 × 12 mm) was located in the center of each of the vertebral bodies L3 and L4. For distal tibia scans, the single voxel (15 × 10 × 10 mm) was placed with the center 34.5 mm proximal to the joint line (Figure 1).

MRS Data Analysis

Prior to MRS data analysis, FSE images were reviewed by a radiologist (TML) to identify fractures or marrow lesions overlapping the MRS voxel. Spine T2 scans were visually inspected for marrow lesions including Modic lesions or Schmorl’s nodes. These were detected based on signal intensity on T2 and on structural abnormalities. Data from voxels

overlapping bone marrow abnormalities were excluded. MRS data were analyzed using in-house software based on previous work[26,27]. Data from each individual channel were processed with frequency and phase corrections. Because peak amplitude may differ among channels, directly averaging all channels may conceal peak contrast. To avoid this, we calculated a combined signal by performing weight-averaging on all channels with the amplitude of the 1.3ppm peak rather than using a fixed weighting. After calculating this combined weight-averaged signal, smoothing (line broadening = 15) was applied on the combined signal to reduce noise.

Spectra data were fitted using a Voigt model with prior knowledge of ppm positions for 7 peaks: 0.9, 1.3, 2.1, 2.8, 4.2, and 5.3 ppm (Figure 2). Specifically, the second peak ("II"; 1.3 ppm) represents saturated lipid, and the sixth peak ("VI"; 5.3 ppm) represents unsaturated lipid. Parameters representing fat content and lipid concentrations were calculated by the following formulas,

$$Total\ Fat\ Content(TFC) = \frac{\sum_{i=1}^{6} a_i}{a_w + \sum_{i=1}^{6} a_i}$$

$$Unsaturated\ Fat\ Content(UFC) = \frac{a_6}{a_w + \sum_{i=1}^{6} a_i}$$

$$Saturated\ Fat\ Content(SFC) = \frac{a_2}{a_w + \sum_{i=1}^{6} a_i}$$

Where a_w is the amplitude of the water peak and a_i is the amplitude of the lipid peaks in ascending order of ppm position.

Statistics

Demographic, laboratory test, and DXA parameters were compared using unpaired two sample t-tests. Generalized estimating equations (GEE) accounting for multiple measurements per person (L3, L4) were used to assess the relationships between T2D status and vertebral MRS outcomes. Linear regression was used to assess the relationships between T2D and tibia MRS outcomes. Standardized beta coefficients were used to evaluate the associations of spine MRS outcomes with serum lipids (total cholesterol, LDL, HDL, and triglycerides). The ratio of total cholesterol to HDL, a lipid biomarker that describes the proportion of "non-HDL" lipids and is commonly used as a marker of cardiovascular disease risk, was also calculated to evaluate its association with MRS outcomes. All analyses were performed on sex-stratified sub-groups and adjusted for age, given the associations of sex and age with BMAT metrics documented in previously published studies[24,25]. Models were further adjusted for statin use, which is associated with serum lipid levels and may potentially impact marrow adiposity. Furthermore, additional adjustment for TFC was performed on the association of UFC and SFC to serum lipids to evaluate associations

of UFC and SFC with serum lipids independent of TFC. A p-value of less than 0.05 was considered as statistically significant. Analyses were performed using STATA version 16 software (StataCorp LP, College Station, TX, USA).

Results

Demographics and Laboratory Assessments

In total, 39 T2D patients and 40 controls were recruited for this study. Three controls were excluded due to prediabetes (based on HbA1c) so 37 controls remained in this study (Table 1). Data from one L3 MRS scan (control) and five L4 MRS scans (three controls and two T2D patients) were excluded due to overlap with bone marrow abnormalities identified on the sagittal fs FSE images. Data from 10 tibia MRS scans (two controls and eight T2D patients) were excluded because of incorrect MRS VOI placement.

BMI, HbA1c, fasting glucose, and triglycerides were significantly higher in T2D participants than in the control group. Total cholesterol, LDL, and HDL were significantly lower in T2D participants than in the control group. No differences between groups were detected in sex, age, race, calcium or vitamin D levels, spine BMD or T-scores. Participants in both groups were on current antilipidemic medication, but the number of participants on current antilipidemic medications was higher in the T2D group. Among these participants on antilipidemic medication, all but two T2D participants used statins. No history of clinical osteoporotic fracture was reported in either group.

In the comparison of the serum lipid levels between sexes, total cholesterol levels were higher in female than male participants within the control group (total cholesterol: p=0.02; Table 1). HDL levels were higher in female than male participants within both control and T2D groups (HDL: control: p=0.0004; T2D: p=0.008). LDL, fasting glucose and HbA1C were not statistically different between sexes within either control or T2D groups (Table 1).

In the subgroup of participants not using antilipidemic medications (n=15 T2D and 30 controls), total cholesterol, LDL, and HDL were significantly lower and triglycerides were significantly higher in T2D participants compared to the control group (Supplementary Table 1). For those participants on antilipidemic medication, a similar pattern was observed although the differences between T2D and control groups were not statistically significant.

MRS BMAT Biomarkers

At the spine, all BMAT biomarkers were significantly lower in male T2D patients compared to male controls in an age-adjusted model (Table 2A; age-adjusted TFC: T2D=76.6±1.2%, Control=81.7±1.4%, p=0.006; UFC: T2D=2.6±0.2%, Control=3.4±0.2%, p=0.004; SFC: T2D=38.8±1.0%, Control=42.4±1.2%, p=0.02). To explore the impact of serum lipids on the relationship between T2D and BMAT biomarkers, we added adjustments for LDL and HDL to the model. For male participants, after adjustment for HDL and LDL, associations of T2D with TFC and T2D with UFC were attenuated and no longer statistically significant. No effect was observed on the association of T2D with SFC. (Table 2B; age-, LDL-, and HDL-adjusted SFC: T2D=38.8±1.0%, Control=42.4±1.3%, p=0.04). For male participants, further adjustment for statin use had little effect on the associations of T2D with BMAT

biomarkers. In females, no significant differences in spine BMAT biomarkers were found between participants with T2D and controls.

At the tibia, no significant differences were found for any BMAT biomarker comparisons between T2D participants and controls, for either males or females (Supplementary Tables 2 & 3).

Associations between Spine Serum Lipid and BMAT Biomarkers

In male T2D participants, significant positive associations of LDL and TC/HDL levels with TFC were found: TFC increased by 2.34% and 1.77 % for every one standard deviation increase in LDL and TC/HDL, respectively (Table 3).

In female T2D participants, significant negative associations between HDL levels and TFC were found: TFC decreased by 3.23% for every one standard deviation increase in HDL.

In male control participants, significant positive associations of TC/HDL and TG levels with SFC were found: SFC increased by 0.87% and 1.34% for every one standard deviation increase in TC/HDL and TG (Table 3).

In female control participants, a significant positive association between HDL and SFC, as well as a significant negative association between TC/HDL and SFC were found: SFC increased by 1.77% and decreased by 3.33% for every one standard deviation increase in HDL and TC/HDL, respectively.

Additional adjustment for BMI did not change any results of the BMAT biomarker vs lipids analyses (data not shown).

Discussion

In this study, we applied MRS and a novel spectrum fitting technique to quantify marrow adiposity and composition in T2D participants and non-T2D controls. Lower total fat content as well as lower unsaturated and saturated fat content at the spine was found in T2D participants compared to controls in age-adjusted models; these differences were significant in men but not in women. In our study cohort, total cholesterol, LDL, and HDL were lower in T2D participants compared to controls. Adjustment for LDL and HDL attenuated the association between T2D status and unsaturated marrow fat, but had little effect on the association between T2D and saturated marrow fat. These associations were not affected by statin use. Further analysis confirmed significant associations between serum lipid levels and BMAT biomarkers. Taken together, our results suggest that bone marrow adiposity and composition are influenced by serum lipid levels as well as T2D status, and these relationships are sex-specific.

Several studies have evaluated bone marrow composition in T2D patients using MRS; however, the methods and results of these previous studies are inconsistent due to differences in sample sizes, cohort demographics, MRS protocols, and BMAT quantification methods. Studies including only females found no differences in marrow adiposity between T2D patients and controls[13–15]. Those including men or mixed-sex cohorts reported

increased adiposity in T2D[11,12]. In our sex-stratified analyses, we found no differences in BMAT biomarkers between female T2D patients and female controls, consistent with prior findings. However, in contrast to prior publications, all BMAT biomarkers were significantly *lower* in male T2D patients than male controls. We hypothesized that clinical characteristics of our participants contributed to lower BMAT biomarkers in male T2D patients. In particular, serum levels of total cholesterol, LDL, and HDL were significantly lower in our T2D patients than in the controls. Although a greater proportion of T2D patients were receiving antilipidemic medication, we found that lipid levels were lower in the T2D patients even when restricting our analysis to participants who were not on antilipidemic medication. To understand the association of T2D status with BMAT parameters independent of serum lipid levels, we added LDL and HDL to our model, and found that this additional adjustment substantially lessened the association of T2D status with UFC. We additionally adjusted for statins, which were used by 62% of T2D participants and only 19% of controls. In this cohort, statin use was associated with T2D status. Statin use is also associated with lower lipid levels. Whether statin use has a direct effect on marrow adiposity is unknown. In our data, statin use did not confound the relationships between T2D status and BMAT biomarkers, as shown in Tables 2A–C, suggesting that statins do not have a direct effect on marrow, but only an indirect effect through lipid levels. (Supplementary Table 4) However, our study was neither designed nor powered to specifically address this question.

Hyperlipidemia has been proposed to be mechanistically related to alterations in marrow adiposity and bone density. Hyperlipidemia promotes marrow adipogenesis through the activation of PPARγ [21]. Preferential differentiation of common precursor cells towards the adipocyte lineage may reduce osteoblastogenesis, thereby decreasing bone quality. In addition to inhibiting osteoblast differentiation, LDL may increase osteoclast differentiation and activity, while HDL may help to alleviate these effects [28–30]. Therefore, high cholesterol, in particular high LDL, can cause imbalance in the regulation of bone formation and resorption, resulting in bone quality decrease[31,32]. While no previous data exist on the relationship between serum lipid levels and marrow adiposity in humans with T2D, Slade et al reported a positive association between serum cholesterol level and marrow adiposity in humans with T1D[22]. This correlation has also been documented in people without diabetes[23]. Our results demonstrate significant associations of serum lipids with BMAT biomarkers of marrow adiposity in T2D. Specifically we found: (1) in the male T2D group, significant positive associations of LDL and TC/HDL levels with total marrow fat content, and (2) in the female T2D group, a significant negative association between HDL and total marrow fat content. These findings support a proposed mechanistic model whereby increased LDL and decreased HDL both contribute to increased marrow adiposity, which in turn results in decreased osteoblastogenesis, increased osteoclastogenesis, and reduced bone quality. A significant association between LDL and marrow adiposity was not found in female T2D patients, suggesting that the major factor causing T2D pathological bone quality reduction may be different between sexes. In contrast to these findings in the T2D patients, in female controls we found a significant *positive* association between HDL and saturated fat content. This contradictory finding could suggest that T2D disrupts healthy relationships between lipid levels and marrow adipose composition. Further investigation is required to clarify this.

Compared to previously published marrow adipose composition results, total fat content values for the participants of this study were relatively high. A possible reason for higher TFC in our study is the advanced age of our cohort (50 to 70 years old), which correlates with higher marrow fat [25]. In addition, participant BMD was low with a high proportion of osteopenic T-scores, which also correlates with higher marrow adiposity. Finally, comparison of marrow adiposity values among studies using varying MRS acquisition and analysis protocols is problematic. Point Resolved Spectroscopy (PRESS) MRS acquisition mode was widely used in previously published research, which differs from the STEAM acquisition used in this study. The shift to STEAM acquisition was motivated by research documenting that fat quantification is inconsistent using PRESS acquisition [33,34]. Further, differences in analysis approaches, including model fitting and variable definitions, may influence the absolute values of fat content calculated in each study.

MRS at the tibia showed no significant differences in BMAT outcomes between T2D and control participants. However, significant concerns about data quality at the tibial spectroscopy site limit the interpretability of these results. Compared to the spine, tibia bone marrow has higher adiposity, and therefore the water peak of the tibia spectrum is relatively small compared to the olefinic peaks. This will introduce errors because the fitting technique requires accurate water peak data. In addition, the width of olefinic peaks in the tibia spectra were smaller than in the vertebral spectra. These factors contributed to poor peak fitting performance in the tibia (Supplementary Figure 1). Further improvement of MRS acquisition and quantification techniques is necessary to increase the accuracy of peak fitting at the tibia; we do not recommend using current techniques for the analysis of marrow composition at the tibia.

This study has several strengths. This is the largest study to date reporting marrow adiposity in both male and female participants with T2D, and the first study to report sex-stratified results and show sex-specific associations between T2D status and BMAT markers of adiposity and composition. This study is also the first to assess associations between serum lipids and BMAT markers in T2D, which may be key to a mechanistic understanding of the impact of T2D on marrow adiposity and bone health. However, this study also has several limitations. First, the cross-sectional study design limits our ability to determine causality. Second, while larger than any previous report of BMAT parameters in T2D men and women, the sample size is modest. Third, we did not include other potentially important clinical factors like adjudicated fracture history, or T2D duration into our statistical models. The study design and small sample size prohibits these additional analyses. Finally, our participants had relatively short T2D duration and well-controlled disease, and therefore may not represent the high fracture risk T2D population in general. Instead, our results may be representative of associations among T2D status, serum lipid levels, and bone marrow adiposity composition in early stage diabetic bone disease.

In conclusion, we utilized MRS to quantify marrow adiposity in participants with T2D and in normoglycemic controls. In males, the quantitative spine BMAT biomarkers total, unsaturated, and saturated fat content were lower in T2D participants. In females, no differences in BMAT biomarkers were detected based on T2D status. Adjustment for LDL and HDL attenuated the association of T2D status with unsaturated marrow adiposity

in males. Further analysis demonstrated significant and sex-specific associations between serum lipid levels and BMAT biomarkers. Taken together, our results suggest that bone marrow adiposity alterations in T2D are sex-specific and dependent on serum lipid levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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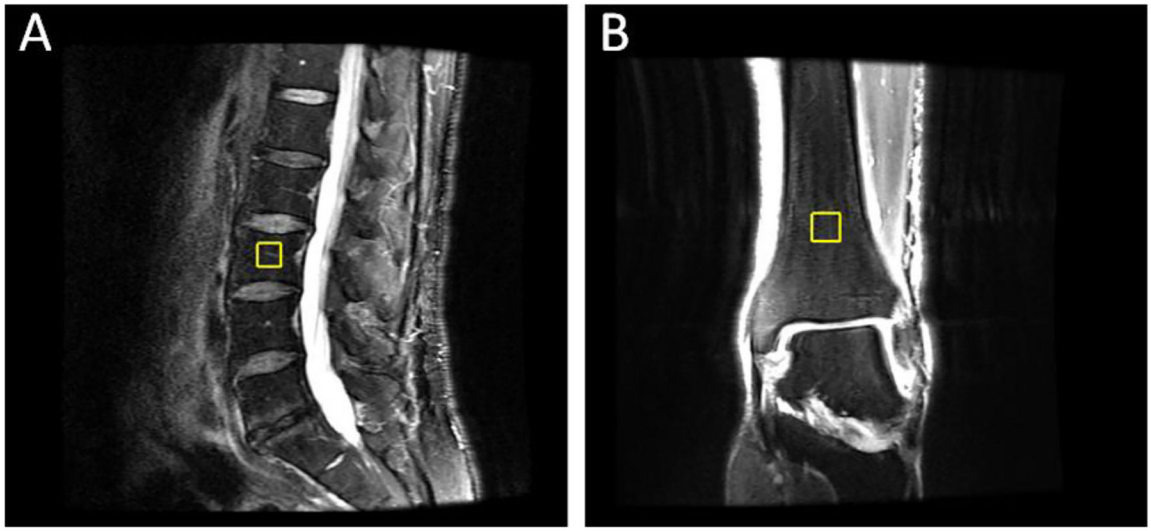

Figure 1:
Example MRS VOI: (a)spine (sagittal view), and (b)distal tibia (coronal view)

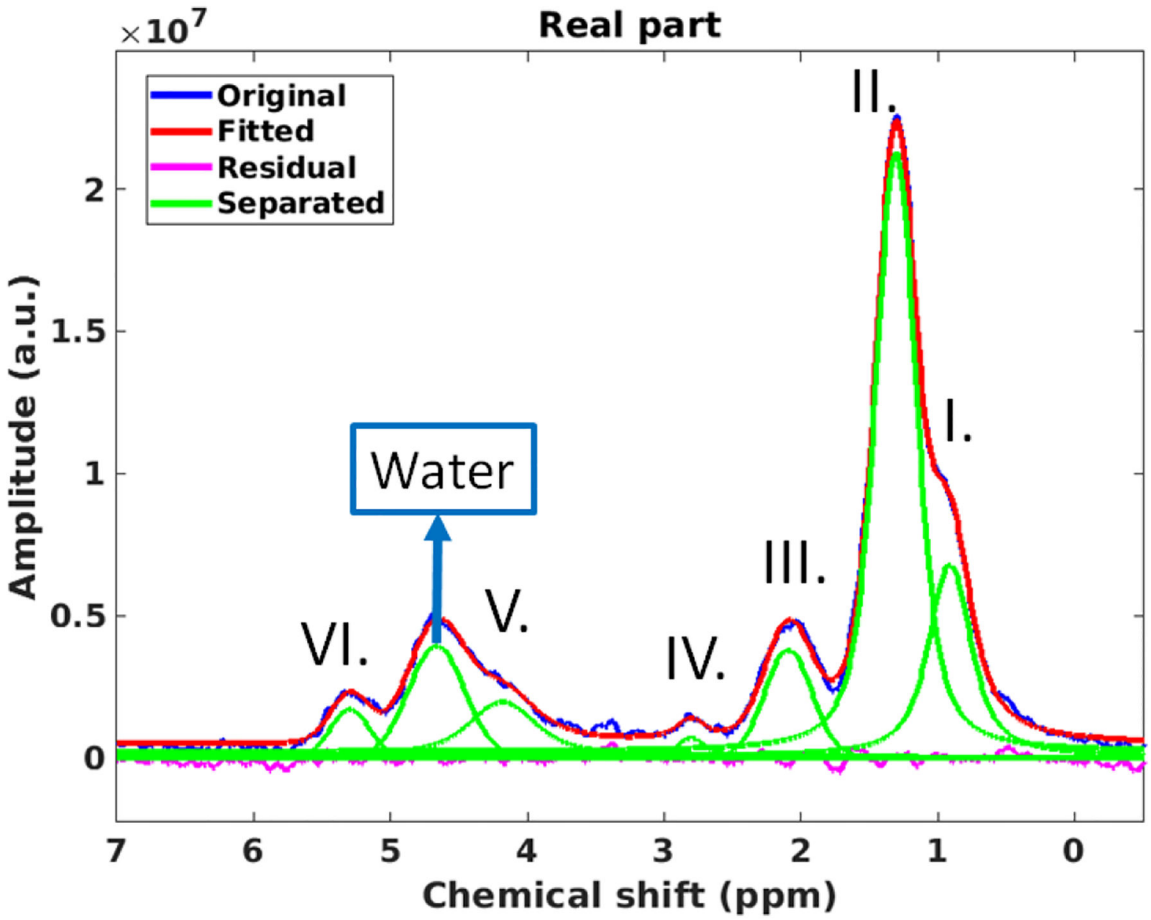

Figure 2:
Example MRS spine spectrum: original spectrum (blue), individual fitted peaks (green), their combined spectrum (red), and the fitting residual (pink). Roman numerals represent the peaks corresponding to olefinic peaks.

Table 1

Participant characteristics. Data shown as counts or mean ± standard deviation. P-values calculated for two-sided unpaired t-tests.

			Male		Female	
	Control	**T2D**	**Control**	**T2D**	**Control**	**T2D**
Demographics						
n	37	39	16	18	21	21
Age (yrs)	62.0±5.4	61.7±5.6	60.9±5.1	59.7±5.7	62.9±5.5	63.4±4.9
Race						
White	26	22	12	13	14	9
Asian	9	12	4	3	5	9
Black	1	4	0	2	1	2
Islander	0	1	0	0	0	1
Not Reported	1	0	0	0	1	0
BMI (kg/m^2)	25.1±3.9	**28.0±4.1** *a*	26.2±4.0	27.7±3.5	24.3±3.8	**28.3±4.7** *c*
Antilipidemic med	7	**24** *a*	4	9	3	**15** *c*
Insulin therapy		8		0		8
T2D duration (yrs)		11.4±7.5		9.9±8.0		12.6±7.0
Laboratory tests						
HbA1c (%)	5.4±0.2	**7.2±1.5** *a*	5.4 ± 0.2	**6.8 ± 1.0** *b*	5.3 ± 0.3	**7.5 ± 1.7** *c*
Fasting glucose (mg/dl)	90.0±16.8	**138.1±52.0** *a*	90.9 ± 23.8	**124.6 ± 34.7** *b*	90.8 ± 9.1	**149.7 ± 61.7** *c*
Total cholesterol (mg/dl)	211.7±45.1	**157.1±38.0** *a*	191.6 ± 52.5	**146.3 ± 34.3** *b*	**227.0 ± 32.0** *b*	**166.3 ± 39.4** *c*
LDL (mg/dl)	122.7±35.2	**83.5±30.9** *a*	114.8 ± 45.0	**78.8 ± 27.1** *b*	128.7 ± 25.0	**87.5 ± 34.0** *c*
HDL (mg/dl)	69.4±22.0	**49.3±14.5** *a*	56.3 ± 13.6	**43.0 ± 9.5** *b*	**79.4 ± 22.1** *b*	**54.8 ± 16.0** *c,d*
Triglycerides (mg/dl)	94.8±48.0	**148.8±66.8** *a*	102.8 ± 45.4	**153.8 ± 73.9** *b*	88.7 ± 50.1	**144.5 ± 61.6** *c*
Calcium	9.4±0.3	9.5±0.3	9.4±0.2	9.5±0.3	9.5±0.3	9.5±0.3
Vitamin D	33.2±9.7	29.4±11.9	31.3±10.5	29.6±12.3	34.7±9.0	29.1±11.9
Serum creatinine (mg/dl)	0.81±0.20	0.88±0.25	0.94±0.13	1.04±0.26	**0.70±0.18** *b*	**0.75±0.12** *d*
DXA						
Spine BMD (g/cm^2)	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1
Spine T-score	−0.7±1.6	−0.6±0.9	−0.6±1.1	−0.8±0.8	−0.7±1.4	−0.5±1.0
Osteopenic	30	28	12	13	18	15

a: statistical significance ($p<0.05$) compared with control group

b: statistical significance ($p<0.05$) compared with male control group

c: statistical significance ($p<0.05$) compared with female control group

d: statistical significance ($p<0.05$) compared with male T2D group

Table 2A.

Spine BMAT composition biomarkers with adjustment for age. Data shown as adjusted mean ± standard error.

BMAT markers	Male					Female				
	Control (n=16)	T2D (n=18)	*P-value*	*β coeff.*	*95% C.I. of difference*	Control (n=21)	T2D (n=21)	*P-value*	*β coeff.*	*95% C.I. of difference*
TFC (%)	**81.7±1.4**	**76.6±1.2**	**0.006**	**−5.12**	**(−8.76, −1.47)**	81.4±1.7	81.0±1.5	0.85	−0.43	(−4.75, 3.90)
UFC (%)	**3.4±0.2**	**2.6±0.2**	**0.004**	**−0.74**	**(−1.24, −0.24)**	3.1±0.3	3.0±0.1	0.74	−0.11	(−0.78, 0.55)
SFC (%)	**42.4±1.2**	**38.8±1.0**	**0.022**	**−3.54**	**(−6.58, −0.50)**	41.0±1.6	42.3±1.2	0.52	1.28	(−2.57, 5.12)

Table 2B.

Spine BMAT composition biomarkers with adjustment for age, HDL, and LDL. Data shown as adjusted mean ± standard error

BMAT markers	Male					Female				
	Control (n=16)	**T2D (n=18)**	***P-value***	***β coeff.***	***95% C.I. of difference***	**Control (n=21)**	**T2D (n=21)**	***P-value***	***β coeff.***	***95% C.I. of difference***
TFC (%)	81.2±1.7	77.0±1.1	0.05	−4.19	(−8.45, 0.08)	82.3±1.9	80.0±2.0	0.48	−2.24	(−8.45, 3.98)
UFC (%)	3.2±0.2	2.8±0.2	0.20	−0.33	(−0.84, 0.17)	2.9±0.2	3.2±0.3	0.37	0.28	(−0.33, 0.88)
SFC (%)	**42.4±1.3**	**38.8±1.0**	**0.04**	**−3.55**	**(−6.92, −0.17)**	41.6±1.6	41.8±1.7	0.93	0.23	(−4.97, 5.42)

Table 2C:

Spine BMAT composition biomarkers with adjustment for age, HDL, LDL, and statin use. Data shown as adjusted mean ± standard error

BMAT markers	Male					Female				
	Control (n=16)	**T2D (n=18)**	***P-value***	***β coeff.***	***95% C.I. of difference***	**Control (n=21)**	**T2D (n=21)**	***P-value***	***β coeff.***	***95% C.I. of difference***
TFC (%)	**81.4±1.6**	**76.9±1.1**	**0.04**	**−4.49**	**(−8.70, −0.27)**	82.3±2.0	80.0±2.1	0.48	−2.33	(−8.84, 4.18)
UFC (%)	3.1±0.1	2.8±0.2	0.21	−0.31	(−0.80, 0.17)	2.9±0.2	3.2±0.3	0.41	0.27	(−0.37, 0.90)
SFC (%)	**42.5±1.2**	**38.8±1.0**	**0.03**	**−3.70**	**(−7.02, −0.39)**	41.6±1.6	41.7±1.7	0.98	0.08	(−5.22, 5.38)

Table 3.

Associations between serum lipid levels and BMAT biomarkers. Data shown as standardized beta coefficients in linear regression models, which describes change in outcome (BMAT biomarker; %) for one standard deviation increase in predictor (serum lipid level).

		BMAT biomarkers		
		TFC *adj. with age, statin use*	**UFC** *adj. with age, TFC, statin use*	**SFC** *adj. with age, TFC, statin use*
Male Control	TC	2.333	−0.038	0.789
	LDL	1.811	−0.058	0.813
	HDL	1.932	0.073	−0.549
	TC/HDL	−0.252	−0.054	**0.873** *
	TG	0.527	−0.036	**1.822** **
Male T2D	TC	2.208	0.291	−1.136
	LDL	**2.337** *	0.233	−0.977
	HDL	−1.946	0.304	−1.494
	TC/HDL	**1.770** **	0.055	−0.074
	TG	1.123	0.049	−0.0300
Female Control	TC	0.697	0.426	−0.505
	LDL	1.632	0.612	−2.762
	HDL	−0.650	−0.032	**1.767** **
	TC/HDL	0.934	0.090	−**3.328** **
	TG	1.138	0.146	−2.108
Female T2D	TC	−0.464	0.176	0.484
	LDL	1.029	0.132	0.644
	HDL	−**3.233** **	0.148	0.470
	TC/HDL	3.390	−0.143	−0.375
	TG	2.124	−0.178	−1.283

TFC = Total fat content; UFC = Unsaturated fat content; SFC = Saturated fat content; TC = Total cholesterol; LDL = low-density lipoprotein; HDL = high density lipoprotein; TG= Triglycerides;

*: p<0.05

**: p<0.01