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Original Contribution

Significance of single and multi-voxel ^{1}H magnetic resonance spectroscopy in the quantification of myocellular lipid in young non-obese Asian Indian males

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ABSTRACT

To prospectively assess intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) using single voxel spectroscopy (SVS) and multi voxel magnetic resonance spectroscopy (MVS) in soleus muscle and correlate results with metabolic variables in non-obese (BMI $<$ 23 kg/m^2) Asian Indian males. Thirty one patients with diabetes (cases) and twelve normoglycaemic subjects (controls) underwent point resolved spectroscopy sequence (PRESS) of soleus muscle using SVS and MVS in a 3 T MRI scanner. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured from MRI images and body composition was measured from dual-energy x-ray absorptiometry (DXA). The mean IMCL from SVS and MVS were 1.6% and 2.6% in cases and 2.3% and 3.4% in controls respectively. The mean EMCL from SVS and MVS were 1.8% and 3% in cases and 1.5% and 3% respectively in controls. A significant correlation between IMCL and total fat mass (rho = 0.42, $p < 0.01$) and total body fat (rho = 0.46; $p < 0.01$) were observed in cases while using the SVS technique and no correlations were found in the MVS technique. The SVS showed significant correlations between total myocellular lipids with VAT and SAT in cases alone. Total myocellular lipids acquired using both techniques showed a significant correlation with BMI, waist circumference, total fat mass, total body fat and truncal fat in cases alone. Quantification of IMCL of soleus muscle using the SVS technique is useful in studying the relationship with metabolic markers in non-obese Asian Indians with diabetes.

1. Introduction

The incidence of Type 2 Diabetes Mellitus (T2DM) has been increasing significantly worldwide, with a higher prevalence in Asian Indians. This increase can be attributed to the rapid modernization and lifestyle changes in Asian Indians [1]. Increased body fat and ectopic fat in Asian Indians predisposes to the early onset of T2DM. Studies have reported associations between insulin sensitivity and T2DM due to excessive accumulation of lipids in the muscle, specifically intra-myocellular lipid (IMCL) [2–4]. Endurance trained athletes accumulate high IMCL to serve as an accessible physical energy storage, which raises important questions about the underlying role of IMCL in the development of metabolic disease [5]. The IMCL content is affected by factors like gender, obesity, diet, fasting, exercise *etc.* [6] and is metabolized during strenuous physical exercise, hence the intensity and duration of exercise are the key factors in determining the reduction of IMCL.

Earlier studies in Asian Indians showed no correlation of IMCL content in the soleus muscle (*S. muscle*) with peripheral insulin sensitivity as measured by the *M*-value index derived from hyperinsulinemic-euglycemic clamp (HEC) studies; however, total body fat was independently associated with IMCL content [3]. This finding depends on the ethnicity of the study population as lower concentrations of IMCL content was observed in the Asian Indians of South India compared to the Asian Indians of Northern India [7]. In a study reported by Maza et al., IMCL content correlated neither with visceral fat nor with insulin sensitivity among African Americans, while positive correlations in these parameters were reported among European Americans [8]. In this context, the measurement of lipid content from specific skeletal muscle is vital for understanding the pathways leading

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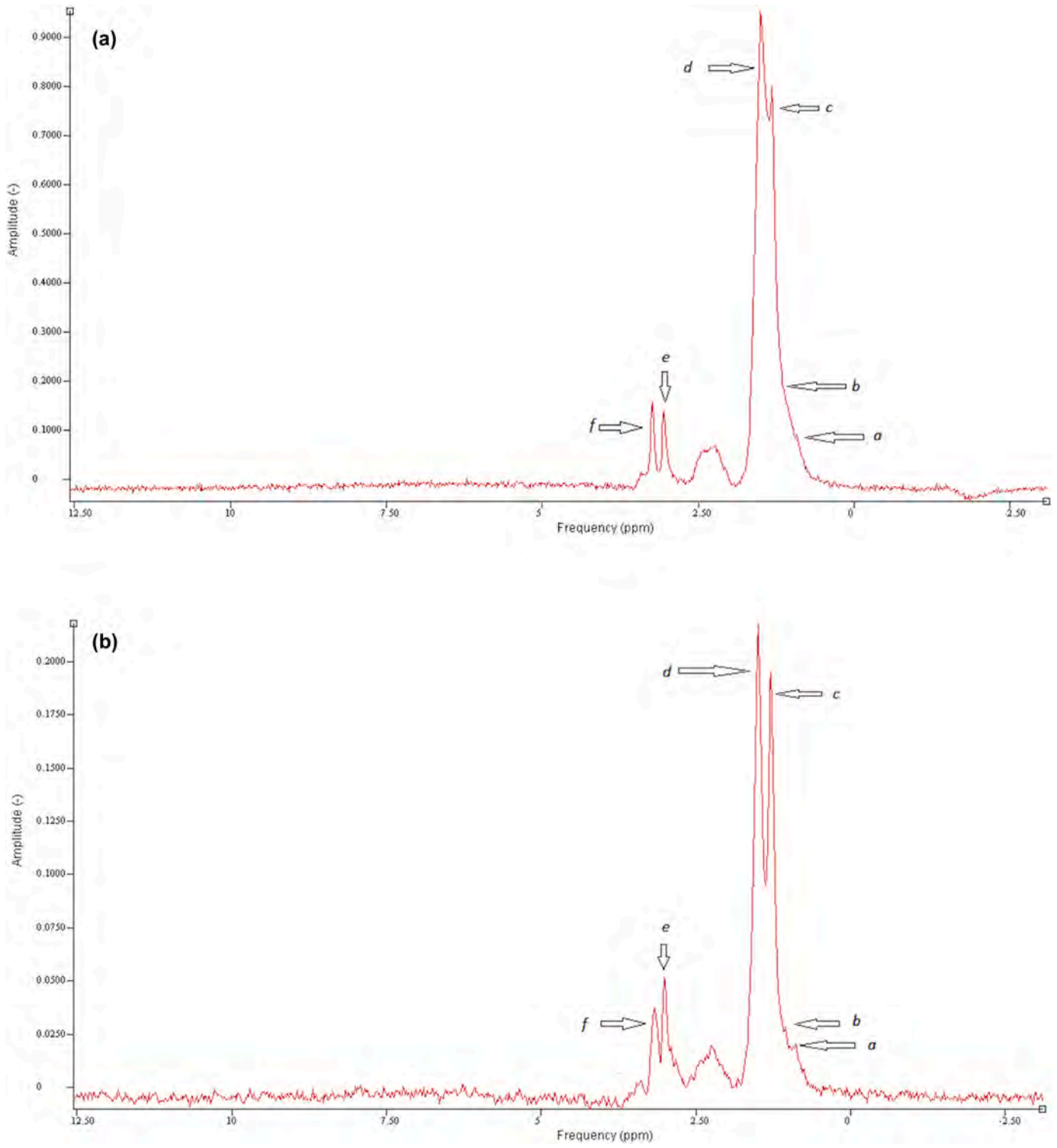

Fig. 1. (a) MR spectra of *S. muscle* acquired using SVS technique. (b) MR spectra of *TA muscle* acquired using SVS technique. Six resonance peaks in both muscles are defined: *(a)* IMCL -CH_3 proton peak at 0.9 ppm, *(b)* EMCL -CH_3 proton peak at 1.1 ppm, *(c)* IMCL-CH_2 proton peak at 1.3 ppm, *(d)* EMCL-CH_2 proton peak at 1.5 ppm, *(e)* total creatinine -CH_3 peak at 3.0 ppm and *(f)* the trimethylamines peak at 3.2 ppm.

to metabolic derangements.

By virtue of its non-invasive and non-radioactive nature, *invivo* magnetic resonance imaging (MRI) and spectroscopy (MRS) provides an approach to quantify lipids in humans and animal models [9]. Measurement of myocellular lipids using MRI or MRS in the skeletal muscle is challenging due to technical variations such as shift in the resonance frequencies, bulk magnetic susceptibility effects and dipolar coupling due to the dipolar interaction between spins in the same molecule [6]. Both single-voxel spectroscopy (SVS) and multi-voxel spectroscopy (MVS) are methods to quantify lipids. These techniques have been reported for measurement of lipids in the *tibialis anterior* (TA) muscle and a few studies in the *S. muscle*. As a Type I fibre muscle, the *S. muscle* requires careful analysis as it experiences a shift of the resonances due to bulk magnetic susceptibility effects [10]. This shift is

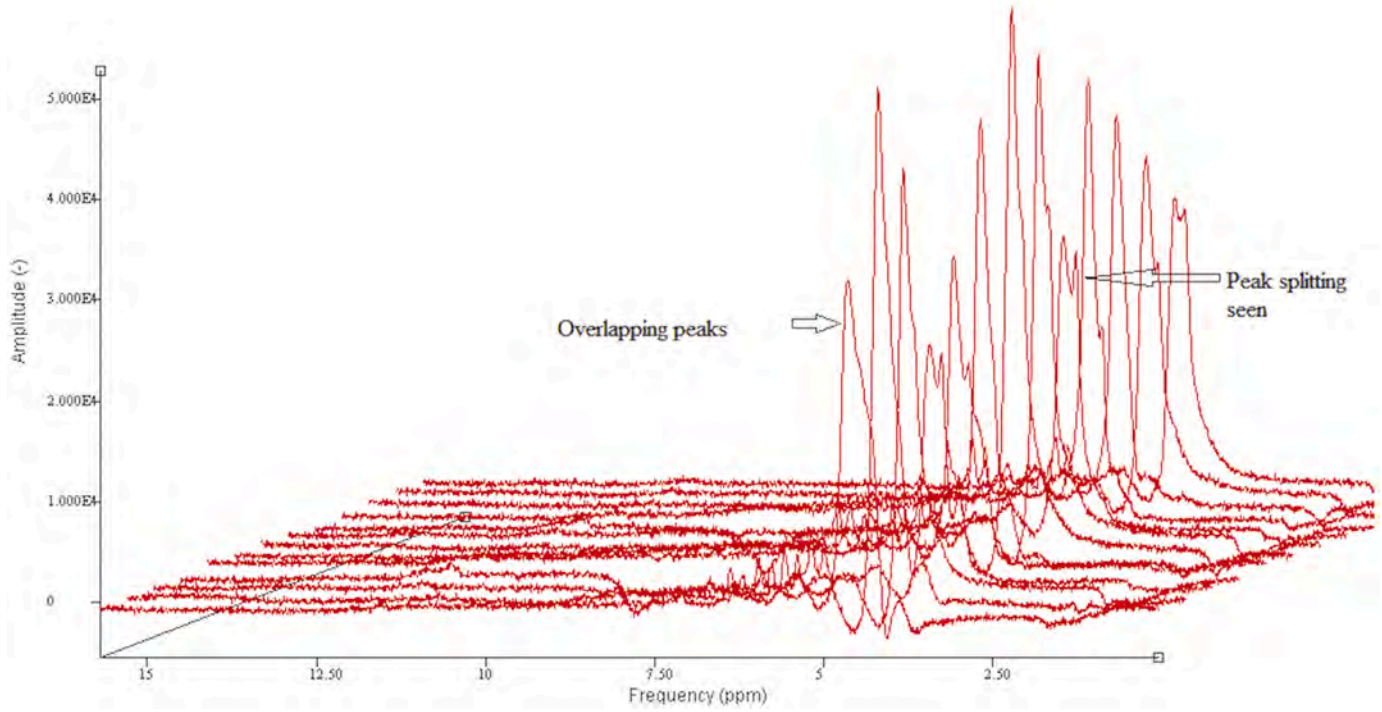

Fig. 2. MR spectra of *S. muscle* acquired using MVS technique. The arrows indicate the splitting of peaks and overlapping peaks.

due to the spatial arrangement of the lipids and not a result of the chemical nature of the muscle. This is not a major concern when quantifying lipid content in the *TA muscle* as the separation of extra myocellular (EMCL) and IMCL peaks can be distinctly visualized in most cases. Compared to SVS, use of MVS offers the feasibility, to choose the desired peak from a cluster of peaks in the muscle involved [11]. Our study evaluates lipid content in the *S. muscle* using both SVS and MVS technique and correlates the results with metabolic variables in non-obese (BMI $<$ 23 kg/m^2) Asian Indian males with and without T2DM. An SVS technique for the *TA muscle* was also performed to correlate results with metabolic variables in this population.

2. Materials and methods

2.1. Patient selection criteria

This study was approved by the Institutional Research Board (IRB) and Ethics committee (IRB no. 7722/2012). A cohort of non-obese (BMI $<$ 23 kg/m^2) Asian Indian males with diabetes (n = 31; cases) and non-obese normoglycaemic controls (n = 12; controls) were recruited with informed written consent. Case and controls were matched for age and BMI.

2.2. Body composition and biochemical investigations

In order to estimate whole body composition, DXA scanner (Hologic, Discovery A, Version 4.2) was used. The fat and lean mass was assessed using the APEX software available in the DXA console. Fasting and post prandial blood samples were analysed for blood glucose, glycosylated haemoglobin (HbA1c) and lipid profile. Glucose was measured by the glucose oxidase-peroxidase method (% CV 3.6). Total cholesterol, low density lipoprotein cholesterol (LDL) and serum triglycerides were measured using enzymatic oxidation and absorbance method as per manufacturers' instructions in enzymatic assay kits supplied by Roche, on Roche Modular P 800 system.

2.3. ^{1}H MRS and MRI acquisition

A 3 T Intera Achieva MR scanner (Philips Medical Systems, Eindhoven, The Netherlands) was used to perform magnetic resonance spectroscopic studies of the *S. muscle* and *TA muscle*. T1-weighted transverse and sagittal cross sections of the *S. muscle* and *TA muscle* were acquired using transmit-receive knee coil. A point-resolved spectroscopy sequence (PRESS) with repetition time (TR)/echo time (TE) of 4000/36 ms, with 32 averages for a voxel size of 15x15x15 mm^3 was used during the SVS technique in both muscles. The MVS data for *S. muscle* was acquired using 2D magnetic resonance spectroscopic imaging (MRSI) technique based on PRESS sequence using iterative shimming for a slice thickness of 20mm with TR/TE = 2000/37 ms. The number of rows was 25 with 5 phase encoding profiles and phase encoding field of view of 45. The number of time domain points were 2048 with a bandwidth of 2 kHz. Both water suppressed and non-water suppressed spectral data was acquired using both techniques. The reproducibility and quality of the spectra were internally assessed and validated using standardized protocols, water was used as an internal reference. For quantification of SAT and VAT, A T1w turbo spin echo (TSE) sequence with TR/TE = 400–510/ 38 ms with a turbo factor of 7 for a 5 mm slice thickness was used to image the entire abdominal region with a similar protocol discussed by Kahl *et al* [12].

2.4. MRS and MRI data processing

In order to correct for relaxation times, T1 and T2 relaxation time values for *S. muscle* and *TA muscle* and muscle water reported by Krssak *et al* was used [13]. Both SVS and MVS data of *S. muscle* were analysed using Java-based magnetic resonance user interface software (jMRUI; Leuven, Belgium). This involved baseline correction; phasing; apodising and fitting using a Gaussian line shape. The residual water was filtered using the Hankel–Lanczos single-variable decomposition (HLSVD) method in the jMRUI software. The metabolite signals were analysed using advanced MR fitting algorithm (AMARES) using prior knowledge in jMRUI software package. For evaluating the area under the peak, each peak was manually selected according to the respective frequency

Table 1
Anthropometry, baseline biochemical, myocellular lipid and body composition of non- obese, normoglycaemic subjects (n = 12) and non–obese subjects with T2DM.

Variables	Controls (n = 12)	Cases (n = 31)	P value
Age (years)	33.3 ± 7.6	32.6 ± 7.4	0.79
Body mass index (kg/m^2)	22.0 ± 3.7	20.6 ± 3.6	0.24
Body surface area (m^2)	1.7 ± 0.1	1.6 ± 0.1	0.19
Waist circumference (cms)	85 ± 11.5	79.5 ± 11.2	0.21
Hip circumference (cms)	89.3 ± 6.3	85.6 ± 8.6	0.25
Waist - hip ratio	0.94 ± 0.07	0.92 ± 0.08	0.48
Biochemical profile			
Fasting blood glucose (mg/dl)	102.6 ± 46.1	206 ± 94.6	0.00
Fasting insulin (U/ml)	7.5 ± 4.9	8.3 ± 6.4	0.78
Glycosylated haemoglobin (%)	5.6 ± 0.7	9.4 ± 2.0	0.00
Total cholesterol (mg/dl)	161.8 ± 30	163 ± 36.1	0.88
Triglyceride (mg/dl)	90.4 ± 32.3	171.4 ± 66.3	0.00
High density lipoprotein cholesterol (mg/dl)	47 ± 11.5	37.1 ± 9.7	0.02
Low density lipoprotein cholesterol (mg/dl)	106 ± 32.2	104 ± 36	0.90
Very low density lipoprotein cholesterol (mg/dl)	20.0 ± 13.4	34.7 ± 12.8	0.05
^{1}H MRS quantification			
S. muscle IMCL (SVS) (%)	2.3 ± 1.3	1.6 ± 1.7	0.19
S. muscle EMCL (SVS) (%)	1.5 ± 0.8	1.8 ± 1.0	0.33
S. muscle IMCL (MVS) (%)	3.4 ± 1.9	2.6 ± 2.0	0.20
S. muscle EMCL (MVS) (%)	3.0 ± 1.1	3.1 ± 1.8	0.92
Total fat in *S. muscle* (SVS) (%)	3.8 ± 1.2	3.4 ± 1.7	0.40
Total fat in *S. muscle* (MVS) (%)	5.3 ± 2.3	4.6 ± 2.1	0.34
TA muscle (EMCL) (%)	1.3 ± 0.6	1.2 ± 0.8	0.79
TA muscle (IMCL) (%)	0.4 ± 0.2	0.7 ± 0.4	0.35
MRI quantification			
Subcutaneous adipose tissue (SAT) cm^3	1187 ± 675	1060 ± 631.7	0.48
Visceral adipose tissue (VAT) cm^3	1061 ± 407* (154, 2190)	828 ± 377.4* (238, 1224)	0.45
Total abdominal adipose tissue (AAT) cm^3	2250 ± 1894* (563, 3844)	1678 ± 1364* (707, 2411)	0.23
VAT/SAT ratio	0.73 ± 0.45* (0.2, 1.3)	0.72 ± 0.56* (0.3, 0.8)	0.84
Body composition (on DXA)			
Total fat (%)	19.5 ± 5.4	17.1 ± 6.0	0.22
Total fat mass (kgs)	12.2 ± 5.9	10.3 ± 5.5	0.32
Total lean mass (kgs)	49.2 ± 5.3	45.1 ± 6.9	0.07
Truncal fat (%)	18.8 ± 7.0	16.1 ± 7.2	0.28
Truncal fat mass (kgs)	5.9 ± 2.9	4.7 ± 3.1	0.24
Truncal lean mass (kgs)	23.7 ± 2.5	21.9 ± 3.6	0.13

Values are presented as mean ± SD/ median (indicated by*) with minimum and maximum values in parentheses. P value < 0.05: statistically significant.

of each metabolite. Fig. 1a and b illustrates the spectrum from SVS technique in *S. muscle* and *TA muscle*. In this spectrum, six resonances were defined: *(a)* IMCL -CH_3 proton peak at 0.9 ppm, *(b)* EMCL -CH_3 proton peak at 1.1 ppm, *(c)* IMCL-CH_2 proton peak at 1.3 ppm, *(d)* EMCL-CH_2 proton peak at 1.5 ppm, *(e)* total creatinine -CH_3 peak at 3.0 ppm and *(f)* the trimethylamines peak at 3.2 ppm. For the MVS technique, 3 distinct peaks from 25 spectral data of *S. muscle* were selected and the average IMCL and EMCL values were included in the analysis (Fig. 2). Similarly, correlations were made for total lipid content (sum of IMCL-CH_2 and EMCL-CH_2) for both techniques.

The T1w MR images were processed using Image J software (NIH Bethesda, USA) version 1.52A for quantification of SAT and VAT [14]. The quantification involved extraction of SAT and VAT separately from the abdominal area covering T12 to L5 vertebral regions with the number of images ranging from 21 to 38 depending upon the size of the patient. The quantification were similar to the methods described in literature [15].

2.5. Statistical analysis

Data were checked for normality and analysed using STATA 11.0 (College station, Texas, USA). Results were presented as mean ± standard deviation. The correlation of dependent variables (IMCL, EMCL, total lipid; SVS and MVS) with anthropometric variables and measures of body composition was performed using Spearman's correlation test. The p value less than 0.05 was considered statistically significant.

3. Results

In this study, significantly higher mean fasting blood glucose, glycosylated haemoglobin and serum triglycerides (all $p < 0.01$) were observed in cases when compared to controls. In contrast, the mean values of high density lipoprotein cholesterol (HDL) were higher in controls compared to cases. No significant differences were observed for anthropometry, myocellular lipids and body composition between cases and controls (Table 1).

3.1. Correlations of myocellular lipids with multiple variables

A significant positive correlation between IMCL of *S. muscle* acquired from the SVS technique with BMI and waist circumference being observed only in cases but not by the MVS technique (Table 2). Similarly, IMCL from SVS technique showed significant correlations with total body fat mass (rho = 0.73, p = 0.00), total body fat (rho = 0.47, p = 0.00), truncal fat % (rho = 0.45, p = 0.00) and truncal fat mass (rho = 0.46, p = 0.00) while the MVS technique revealed a significant correlation only in percentage truncal fat exclusively in cases.

The EMCL of *S. muscle* acquired from the MVS technique showed a significant correlation with BMI in both controls (rho = 0.56, p = 0.04) and in cases (rho = 0.38, p = 0.03) which was not observed in the SVS technique. Significant correlations were found between total fat mass, total fat, truncal fat mass and truncal fat only in cases in both techniques as shown in Table 3. The IMCL and EMCL in *TA muscle* acquired using an SVS technique showed a significant correlation with total body fat mass, total body fat and truncal fat only in cases as shown in Table 4.

In order to calculate total lipid content in *S. muscle*, the sum of IMCL and EMCL were considered in both techniques. As shown in Table 5, both techniques showed a significant correlation between total lipid content with BMI, total fat mass, total body fat, truncal fat and truncal fat mass particularly in cases. The total lipid acquired using the SVS technique showed significant correlations with IMCL of the *TA muscle*, VAT, total AAT and VAT/SAT ratio (rho = 0.52, p = 0.00; rho = 0.49, p = 0.01; rho = 0.46, p = 0.02 and rho = 0.4, p = 0.04) in cases whereas this was not observed in the MVS technique. Fig. 3 (a-d) shows correlation graphs of IMCL with total body fat percentage of *S. muscle* for both cases and controls using both SVS and MVS techniques.

4. Discussion

To the best of our knowledge, this is the first case-control study on the comparative analysis of SVS and MVS for quantification of IMCL and EMCL from *S. muscle* in a 3 T MR scanner and its correlation with measures of anthropometry, body composition and biochemical parameters in young, non-obese, Asian Indian males, with and without diabetes. Quantification of myocellular lipids (especially IMCL) plays a vital role in the clinical management of diabetes and metabolic diseases [16]. Asian Indians have an increased propensity to store lipids at ectopic sites such as liver [1] and *S. muscle* [17]. They also have a unique phenotype featured by excess body fat, increased truncal fat and lower lean body mass even at low BMI as compared to White Caucasians [18]. One of the problems related to quantification of lipids in *S. muscle* is the

Table 2
Correlation of IMCL from *S. muscle* acquired using SVS and MVS with independent variables.

Variables	IMCL of *S. muscle* (SVS) in % water resonance peak intensity				IMCL of *S. muscle* (MVS) in % water resonance peak intensity			
	Controls(n = 12)		Cases (n = 31)		Controls(n = 12)		Cases (n = 31)	
	rho	*P* value	rho	*P* value	rho	*P* value	rho	*P* value
Age (years)	0.13	0.65	0.33	0.06	0.08	0.82	0.48	0.00
Body mass index (kg/m^2)	0.48	0.08	0.48	0.00	0.20	0.60	0.25	0.16
Body surface area (m^2)	0.33	0.25	0.26	0.14	0.10	0.79	0.12	0.51
Waist circumference (cms)	0.26	0.48	0.49	0.00	0.20	0.60	0.32	0.09
Hip circumference (cms)	0.20	0.59	0.31	0.09	0.01	0.96	−0.01	0.95
Waist –hip ratio	0.26	0.48	0.24	0.21	0.01	0.80	0.33	0.06
TA muscle (IMCL) (%)	0.51	0.07	0.35	0.05	0.32	0.30	0.30	0.10
TA muscle (EMCL) (%)	0.28	0.33	0.29	0.10	−0.25	0.41	0.20	0.26
SAT (cm^3)	0.46	0.13	0.36	0.08	0.39	0.18	−0.08	0.70
VAT (cm^3)	0.25	0.41	0.37	0.08	0.17	0.57	0.13	0.51
Total AAT (cm^3)	0.29	0.35	0.41	0.05	0.31	0.28	0.05	0.80
VAT/SAT ratio	0.35	0.22	0.24	0.25	0.18	0.53	0.19	0.36
Total fat mass (kgs)	0.73	0.00	0.73	0.00	0.41	0.18	0.31	0.09
Total body fat (%)	0.32	0.30	0.47	0.00	0.31	0.31	0.32	0.07
Truncal fat (%)	0.35	0.25	0.45	0.01	0.23	0.45	0.36	0.04
Truncal fat mass (kgs)	0.27	0.37	0.46	0.02	0.37	0.23	0.32	0.08
Total Lean mass (kgs)	0.01	0.96	0.32	0.08	0.34	0.27	0.13	0.49

* - % water resonance peak intensity; *P* value < 0.05: Statistically significant.

Table 3
Correlation of EMCL from *S. muscle* acquired using SVS and MVS with independent variables.

Variables	EMCL of *S. muscle* (SVS) in % water resonance peak intensity				EMCL of *S. muscle* (MVS) in % water resonance peak intensity			
	Controls (n = 12)		Cases (n = 31)		Controls (n = 12)		Cases(n = 31)	
	rho	*P* value	rho	*P* value	rho	*P* value	rho	*P* value
Age (years)	−0.11	0.77	0.33	0.08	0.19	0.60	0.35	0.06
Body mass index (kg/m^2)	−0.30	0.45	0.02	0.89	0.56	0.04	0.38	0.03
Body surface area (m^2)	−0.30	0.45	−0.09	0.64	0.21	0.57	0.22	0.25
Waist circumference (cms)	−0.30	0.45	0.06	0.76	0.51	0.16	0.38	0.03
Hip circumference (cms)	−0.39	0.33	−0.11	0.55	0.32	0.39	0.07	0.69
Waist –hip ratio	−0.38	0.35	0.29	0.13	0.51	0.16	0.48	0.01
TA muscle (IMCL) (%)	−0.45	0.13	0.23	0.20	0.44	0.15	0.04	0.82
TA muscle (EMCL)(%)	0.53	0.07	0.27	0.13	0.40	0.19	0.16	0.37
SAT (cm^3)	−0.44	0.13	−0.09	0.66	0.41	0.15	0.21	0.31
VAT (cm^3)	−0.11	0.71	0.18	0.38	0.35	0.23	0.28	0.16
Total AAT (cm^3)	−0.26	0.37	0.02	0.91	0.36	0.22	0.23	0.26
VAT/SAT ratio	−0.12	0.68	0.23	0.26	0.35	0.22	0.21	0.32
Total fat mass (kgs)	0.34	0.26	0.39	0.03	0.34	0.26	0.39	0.03
Total fat %	0.23	0.46	0.37	0.04	0.23	0.46	0.37	0.04
Truncal fat mass (kgs)	0.22	0.48	0.39	0.03	0.22	0.48	0.39	0.03
Truncal fat (%)	−0.39	0.19	0.40	0.02	0.25	0.41	0.40	0.02
Total body fat (%)	−0.51	0.08	0.31	0.09	0.23	0.46	0.37	0.04
Total lean mass (kgs)	−0.20	0.52	−0.25	0.06	0.06	0.83	0.31	0.09

P value < 0.05: Statistically significant.

oblique orientation of the muscle (Type 1 fibre) when the leg axis is placed parallel to the static magnetic field and placement of voxel in the field of view, as maximum signal intensity is obtained when the external magnetic field is parallel to the muscle fibres. For a few subjects, overlapping of IMCL and EMCL peaks was observed while performing spectroscopy using both techniques; however this issue was circumvented when the post processing algorithm was used in peak separation and quantification. In case of *TA muscle* (Type I/II fibre), the fibre orientation is along the main magnetic field showing distinct separation of lipid compartments, hence, only SVS technique was used.

The IMCL of *S. muscle* quantified using *SVS* technique provided significant correlation with BMI, waist circumference, total body fat and truncal fat. This was not observed from the *MVS* technique. When total lipid content was considered, significant correlation with anthropometry and body composition in cases were observed when either techniques were employed as shown in Table 5. It has been reported that the Asians with low BMI tend to accumulate VAT more than other ethnic populations [19]. In this study, a strong correlation of total lipid content with VAT, AAT and VAT/SAT ratio was observed only in cases when SVS technique was employed. The EMCL of *S. muscle* correlated well with total fat and truncal fat only in cases when both techniques were considered but did not show any relationship with other metabolic markers as compared to IMCL. Hence, this study indicates the importance of IMCL in understanding metabolic diseases and quantification using an SVS technique.

In a study conducted on healthy lean and obese subjects to compare SVS *vs* MVS in a 1.5 T MR scanner on a small sample size in *TA muscle*, Shen et al., reported on the flexibility of the MVS approach, specifically its minimal setup time in post-acquisition voxel selection, as compared to the SVS technique. In their study cohort, no significant correlation

Table 4
Correlation of IMCL and EMCL of *TA muscle* acquired using SVS with independent variables.

Variables	IMCL of *TA muscle* in % water resonance peak intensity				EMCL of *TA muscle* in % water resonance peak intensity			
	Controls (n = 12)		Cases (n = 31)		Controls (*n* = 12)		Cases (*n* = 31)	
	rho	*P* value	rho	*P* value	rho	*P* value	rho	*P* value
Age (years)	−0.50	0.16	0.45	0.01	−0.24	0.52	0.44	0.01
Body mass index (kg/m^2)	−0.13	0.73	0.10	0.60	−0.51	0.15	0.26	0.17
Body surface area (m^2)	0.28	0.46	0.16	0.39	−0.06	0.86	0.27	0.15
Waist circumference (cms)	−0.06	0.86	0.19	0.30	−0.53	0.13	0.39	0.03
Hip circumference (cms)	0.10	0.79	0.04	0.83	−0.72	0.02	0.20	0.29
Waist -hip ratio	−0.06	0.86	0.32	0.09	−0.53	0.13	0.42	0.02
S. muscle IMCL (SVS) (%)	0.68	0.01	0.39	0.02	0.34	0.26	0.31	0.08
S. muscle EMCL (SVS) (%)	−0.45	0.13	0.23	0.20	0.53	0.07	0.27	0.13
S. muscle IMCL (MVS) (%)	0.32	0.30	0.30	0.10	−0.25	0.41	0.20	0.26
S. muscle EMCL (MVS) (%)	0.44	0.15	0.04	0.82	0.40	0.19	0.16	0.37
SAT (cm^3)	−0.03	0.90	0.15	0.47	−0.29	0.33	0.24	0.23
VAT (cm^3)	−0.37	0.20	0.34	0.09	−0.06	0.83	0.27	0.19
Total AAT (cm^3)	−0.31	0.30	0.31	0.13	−0.21	0.47	0.33	0.11
VAT/SAT ratio	−0.29	0.32	0.37	0.06	−0.01	0.97	0.21	0.30
Total fat mass (kgs)	0.09	0.77	0.40	0.02	−0.28	0.36	0.56	0.00
Total body fat %	0.02	0.93	0.43	0.01	−0.47	0.11	0.57	0.00
Truncal fat (%)	−0.08	0.80	0.47	0.00	−0.30	0.33	0.58	0.00
Truncal fat mass (kgs)	−0.01	0.97	0.35	0.05	−0.45	0.13	0.46	0.00
Total lean mass (kgs)	0.27	0.38	0.15	0.40	−0.29	0.35	0.24	0.18

P value < 0.05: Statistically significant.

Table 5
Correlation of total myocellular lipids from soleus muscle acquired using SVS and MVS with independent variables.

Variables	Total myocellular lipids of *S. muscle* (SVS) %				Total myocellular lipids of *S. muscle* (MVS) %			
	Controls (n = 12)		Cases (n = 31)		Controls (n = 12)		Cases (n = 31)	
	rho	*P* value	rho	*P* value	rho	*P* value	rho	*P* value
Age (years)	−0.25	0.51	0.62	0.00	0.21	0.57	0.55	0.00
Body mass index (kg/m^2)	−0.35	0.35	0.55	0.00	0.63	0.06	0.38	0.04
Body surface area (m^2)	−0.18	0.63	0.33	0.08	−0.28	0.46	0.18	0.34
Waist circumference (cms)	−0.30	0.43	0.56	0.00	0.71	0.02	0.41	0.02
Hip circumference (cms)	−0.62	0.07	0.30	0.11	0.42	0.25	0.04	0.80
Waist –hip ratio	−0.30	0.43	0.49	0.00	0.71	0.02	0.49	0.00
TA (IMCL) (%)	0.44	0.14	0.52	0.00	0.41	0.18	0.20	0.27
TA (EMCL) (%)	0.52	0.07	0.35	0.05	−0.02	0.93	0.20	0.28
SAT (cm^3)	0.14	0.64	0.36	0.08	0.60	0.02	0.09	0.67
VAT (cm^3)	0.07	0.79	0.49	0.01	0.36	0.21	0.21	0.31
Total AAT (cm^3)	0.04	0.87	0.46	0.02	0.53	0.06	0.16	0.43
VAT/SAT ratio	0.13	0.65	0.40	0.04	0.36	0.22	0.18	0.38
Total fat mass (kgs)	−0.01	0.98	0.56	0.00	0.38	0.21	0.41	0.02
Total body fat %	−0.17	0.58	0.56	0.00	0.25	0.42	0.41	0.02
Truncal fat (%)	−0.06	0.84	0.55	0.00	0.23	0.45	0.45	0.01
Truncal fat mass (kg)	−0.20	0.51	0.50	0.00	0.34	0.26	0.37	0.03
Total lean mass (kgs)	−0.16	0.60	0.26	0.16	0.25	0.42	0.22	0.23

P value < 0.05: Statistically significant.

was reported with measures of body composition except for BMI and waist circumference with EMCL, despite distinct separation of IMCL and EMCL peaks in *TA muscle* [20]. In order to understand the role of *TA muscle* in metabolic diseases; IMCL and EMCL quantified using SVS technique in our study showed significant correlations with total fat mass, total body fat and truncal fat only in cases. When both muscles are considered, the *S. muscle* was considered to be vital for myocellular lipid quantification as it displayed a greater relationship with metabolic parameters than *TA muscle* in this non-obese diabetic Asian population. Our study involved the use of short TE in both spectroscopic techniques. Further studies with long TE and no water suppression would provide better detection of myocellular lipids as reported in literature [21,22].

In conclusion, we found that the IMCL of *S. muscle* measured by *SVS* technique showed stronger relationship with metabolic markers in a non-obese diabetic Asian Indian indicating the advantage of the technique when compared to *MVS*. When the total lipid content is to be quantified, either of the techniques may be considered. The *S. muscle* also offered improved correlations with metabolic parameters when compared to the *TA muscle*.

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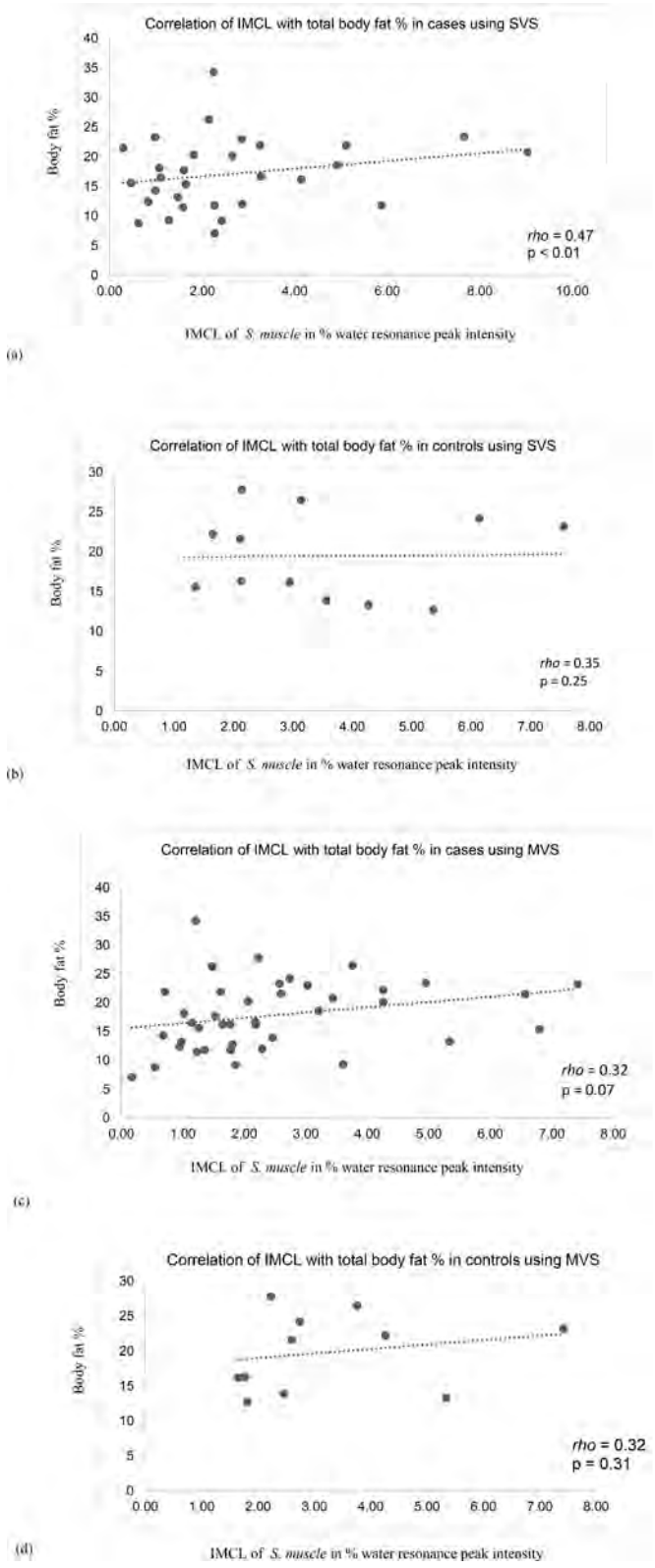

Fig. 3. Figure 3 (a-d) shows correlation graphs of IMCL with total body fat percentage of S. muscle for both cases and controls using both SVS and MVS techniques.

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