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Deep subcutaneous adipose tissue lipid unsaturation associates with intramyocellular lipid content

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ABSTRACT

Background. Obese twins have lower saturated and higher long-chain polyunsaturated fatty acids (FA) in subcutaneous adipose tissue (SAT) compared to their lean monozygotic (MZ) co-twin. Whether this holds for metabolically distinct deep (DSAT) and superficial (SSAT) depots is unknown. Here we use non-invasive magnetic resonance spectroscopy (MRS) to measure the FA unsaturation in body mass index (BMI) discordant MZ twins in DSAT and SSAT and their relationship to ectopic fat content and body fat distribution. The main finding is further confirmed in an independent cohort using standardized measurement times.

Methods. MRS and magnetic resonance imaging were used to measure DSAT and SSAT unsaturation and their relationship to intramyocellular lipids (IMCL), hepatocellular lipids (HCL) and the amount of subcutaneous (SAT) and visceral adipose tissue (VAT) in 16 pairs of healthy monozygotic twins (MZ) discordant for BMI. A second independent cohort of 12 healthy volunteers was used to measure DSAT unsaturation and IMCL with standardized measurement time. One volunteer also underwent repeated random measurements of DSAT unsaturation and IMCL.

Abbreviations: MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; MZ, monozygotic; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; IMCL, intramyocellular lipids; FA, fatty acid; PUFA, polyunsaturated fatty acid; SAT, subcutaneous adipose tissue (volume around waist); VAT, visceral adipose tissue (volume around waist); HCL, hepatocellular lipids; PRESS, point resolved spectroscopy sequence; NEFA, non-esterified fatty acid; BMI, body mass index; TA, tibialis anterior; T₁, longitudinal relaxation time; T₂, transverse relaxation time; T1/2, half-life time constant; TE, echo time; UI, unsaturation index (olefinic/methylene); VOI, volume-of-interest; IPR, intra-pair resemblance.

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Results. In accordance with biopsy studies SSAT unsaturation was higher in the heavier twins ($15.2 \pm 1.0\%$ vs. $14.4 \pm 1.5\%$, $P = 0.024$) and associated with SAT volume ($R = 0.672$, $P = 0.001$). DSAT unsaturation did not differ between twins (11.4 ± 0.8 vs. 11.0 ± 1.0 , $P = 0.267$) and associated inversely with IMCL content ($R = -0.462$, $P = 0.001$). The inverse association between DSAT unsaturation and IMCL was also present in the participants of the second cohort ($R = -0.641$, $P = 0.025$) and for the repeated sampling at random of one person ($R = -0.765$, $P = 0.027$).

Conclusions. DSAT and SSAT FA unsaturation shows distinct associations with obesity and IMCL in MZ twins, reflecting compartment-specific metabolic activities. The FA unsaturation in the DSAT depot associates inversely with IMCL content, which raises the possibility of cross talk between the DSAT depot and the rapid turnover IMCL depot.

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1. Introduction

Interest in adipose tissue fatty acid (FA) composition has mainly focused on its use as a marker of dietary fat intake, while other factors contributing to the FA profile of adipose tissue have received less attention. The overall degree of unsaturation of fat seems to vary between different fat depots [1], which indicates tissue-specific non-dietary modification of FA composition and likely arises from differences in the metabolic activity. How the metabolic activity of the tissue impacts the FA pattern is still unclear, but may include tissue-specific FA synthesis, desaturation and selective mobilization [2–4].

Adipose tissue is now considered an active endocrine organ that modulates interorgan crosstalk through adipokines [5]. Recently, also FA has been defined as adipokines or “lipokines” that can modulate liver and muscle function [6]. Thus, altered FA adipose tissue composition may lead to changes in the non-esterified FA (NEFA) secretion patterns and in turn exert effects throughout the body. Although adipose tissue triglycerides are considered the long-term energy reserves of the body, adipose tissue fat storage, lipolytic activity and blood flow are regulated on an intraday basis [7,8]. During the postprandial period, lipolysis is suppressed and fat storage upregulated, while even a single bout of exercise increases lipolytic release of NEFA from adipose tissue [9]. Adipose tissue fat turnover is decreased in obesity, along with an increase in fat storage and decrease in lipolytic activity, rendering adipocyte turnover a potential target for treating metabolic diseases [10].

NEFA released from adipose tissue during exercise is mainly taken up by skeletal muscle, where they are oxidized or stored as intramyocellular lipids (IMCL). Interestingly, IMCL are rapidly depleted by a single bout of exercise [11], associate with insulin resistance [12], but are also paradoxically increased in insulin-sensitive athletes [13]. Insulin resistance is an early sign of obesity related metabolic disorders and is associated with increased visceral and ectopic fat accumulation, in IMCL as well as in the liver. Impaired suppression of adipose tissue NEFA release by insulin is thought to lead to ectopic fat accumulation [9].

Studies on human adipose tissue FA composition have almost exclusively been conducted on adipose tissue samples obtained by needle aspiration biopsy. This introduces limitations due to variable tissue sampling. Recently, magnetic resonance spectroscopy (MRS) has emerged as a non-invasive alternative for measuring adipose tissue unsaturation [14]. In addition to

allowing repeated sampling of the same location, MRS can also distinguish between deep (DSAT) and superficial subcutaneous adipose tissue (SSAT), which are separated by a MRI visible fascia and exhibit marked differences in FA composition [15] and metabolic activity [16]. Of note, traditional adipose tissue sampling techniques generally have not differentiated between these two subcutaneous adipose depots [17].

Body fat distribution has an important genetic component [18,19], which may also hold true for FA composition in adipose tissue [20]. Monozygotic twin pairs (MZ) discordant for body mass index (BMI) provide a means to study environmental factors affecting human metabolism, while controlling for genetic influences. As the MZ twin pairs have the same genomic sequence, any observed phenotype differences can be mostly attributed to external, acquired factors, ranging from somatic mutations, to diet, microbiome and physical activity. Our previous twin study found lower saturated stearic acid (18:0) and higher long-chain polyunsaturated arachidonic acid (20:4n-6) in the adipose tissue of obese compared to their lean monozygotic (MZ) co-twin [21]. This study, however, did not differentiate between the DSAT and SSAT compartments.

The present study now examined whether twins from MZ pairs discordant for BMI also display depot specific differences in adipose tissue unsaturation (DSAT and SSAT), and how the unsaturation relates to body fat distribution and ectopic fat. Additional measurements were performed to corroborate the findings in a second cohort with measurements standardized to the fasting state.

2. Materials and Methods

2.1. Participants

Two separate cohorts were recruited, an initial twin cohort and a second cohort for further testing and confirming the findings obtained in the twin cohort in a more general population and in the fasted state. The twin cohort consisted of 32 MZ twins (16 males, 16 females, 16 pairs) aged 33–36 years specifically recruited for discordance in BMI, drawn from a large cohort of twins born 1975–1979 in Finland (the FinnTwin16 study) [22]. None of the twins had any comorbidities or medications. The twins underwent a full measurement protocol for MRI and MRS (see details below), with the exception of SSAT unsaturation, which could not be

measured in 6 twin pairs, due to timing restrictions. The twin study was approved by the Hospital District of Helsinki and Uusimaa Ethics Committee. The second cohort consisted of 12 volunteers (11 males, 1 female) age 40.8 ± 15.0 years and BMI 24.9 ± 2.2 kg/m², who were recruited through newspaper advertisements and word of mouth. Inclusion criteria were absence of any known disease or medication, and report of an active lifestyle. The second cohort was approved by the Ethics Board of Heinrich-Heine University Düsseldorf, Germany.

All studies adhered to the ethical guidelines of the Declaration of Helsinki. All participants gave their written informed consent to the respective study protocols. All participants were instructed to maintain their usual dietary behavior, and to restrain from strenuous physical activity for 2 days prior to the measurements.

2.2. Magnetic Resonance Spectroscopy and Imaging

2.2.1. Twin Study

For the twin study, a clinical 1.5-T MR imager (Avanto, Siemens, Erlangen, Germany) was used for acquiring localized proton spectra from abdominal deep subcutaneous adipose tissue (DSAT) at the level of the umbilicus, from superficial subcutaneous adipose tissue (SSAT) of the calf, from tibialis anterior muscle (TA), and from liver (HCL). Also the distribution of mid-body subcutaneous (SAT) and visceral adipose tissue (VAT) was measured during the same session. The measurement setups have been previously described for DSAT unsaturation [15] and IMCL [11,12]. In short, a PRESS (Point Resolved Spectroscopy Sequence) sequence was used for localization, with volume-of-interest (VOI) placement on T₁-weighted localizer images avoiding veins and contamination from surrounding tissue. DSAT and SSAT were measured using long echo time (TE) = 200 ms with 16 averages without water suppression, while IMCL was measured using short TE (30 ms) with 64 averages with water suppression. The repetition time was set to 3000 ms for all spectra obtained at 1.5 T. The SSAT spectra were obtained from the right calf from ten of the sixteen twin pairs (10 females, 10 males). Due to timing restrictions, the MRI and MRS measurements were mainly performed in the evening between 4 pm and 10 pm, after at least 4 hours of fasting before the measurements.

HCL spectra and SAT and VAT volumes were quantified as previously described [23]. In short, hepatocellular lipids (HCL) were measured using the PRESS sequence with and without water suppression, with the HCL content measured from the CH₂ signal relative to the water signal and expressed as a fat fraction. SAT and VAT were measured using a T₁-weighted MRI sequence involving a 16 cm stack centered at the L4/5 intervertebral disk. SAT and VAT content were analyzed from the images using the SliceOmatic software (v 4.3).

2.2.2. Second Cohort Study

For these studies, a 3.0 T MR imager (Achieva, Philips, Best, The Netherlands) was used to measure spectra from the DSAT and IMCL in TA only. All spectra were acquired with the same protocol as for the twin cohort, except that the repetition time was set to 4000 ms to account for the possible increase in T₁ due to the higher field strength. All measurements were performed in the morning after an overnight fast.

One weight stable individual (male, 37 years, BMI = 25 kg/m²) also completed a random sampling study, where the DSAT and IMCL measurements were performed 8 times on separate random days over the course of 1 year, to study intra-individual day-to-day variation. The random sampling measurements were otherwise identical to the other studies with the exception that the time of day of the measurements was also random and that no restrictions regarding dietary intake or physical activity were given.

2.3. Analysis

Spectra obtained from adipose tissue and liver were analyzed using the jMRUI 3.0 software (<http://www.mrui.uab.es/mrui/>) [24]. Spectra obtained from skeletal muscle were analyzed using LCModel [25]. The adipose tissue spectra were used to measure FA unsaturation by using the ratio of olefinic to methylene fat resonances and expressed as percent. IMCL content was calculated as the intensity of the IMCL methylene (1.3 ppm) resonance referenced to total creatine.

Statistical analysis was performed using SPSS Statistics version 20.0.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and Stata 14 (StataCorp LP, College Station TX, USA). The difference between twin pairs was tested using the paired Student's t-test. Associations between variables were analyzed by univariate correlation analyses using partial correlation using Pearson's product-moment correlation coefficient adjusted for clustered sampling to correct for twin relatedness [26]. In the case of delta values Spearman's rank correlation coefficient was used for unadjusted correlation coefficients. When using Pearson's product-moment correlation coefficient, non-normally distributed data were logarithmically transformed; this was the case for SAT volume and IMCL content in the twin study. Normality of the distribution was tested using the D'Agostino–Pearson test. The Benjamini–Hochberg procedure was used to further control the false discovery rate of P-values obtained with multiple comparisons.

MZ pairs were compared for lean vs. heavy twin differences and twin intra-pair resemblance (IPR), i.e. correlation between twins with (R^2). The association between unsaturation in adipose depots and body fat distribution and ectopic fat was determined for the pooled twin data and for the intra-pair difference (Δ = heavy twin – lean twin). Correlations were calculated between BMI, SAT, VAT, HCL, IMCL and adipose tissue unsaturation in DSAT and SSAT. The difference between DSAT unsaturation and SSAT unsaturation was also tested with a paired t-test. A P-value of 0.05 was considered to indicate statistical significance.

3. Results

3.1. Relationship Between Adipose Tissue Unsaturation and Fat Distribution in Twins

The heavier twins were on average 15.5 ± 12.8 kg (median 13.2 kg) heavier than their leaner counterparts. The heavier twins also displayed increased HCL, VAT, and SAT (Table 1). SSAT unsaturation was higher in the heavier twin ($15.2 \pm 1.0\%$ vs. $14.4 \pm 1.5\%$, $P = 0.024$) and associated to SAT volume ($R = 0.549$,

Table 1 – Characteristics of the heavy vs. lean twins.

	Heavy twin	Lean twin	Paired t-test	IPR
Fasting glucose (mmol/L)	5.4 ± 0.4	5.2 ± 0.4	<i>P</i> = 0.107, <i>n</i> = 16	17%
Fasting insulin (μIU/mL)	8.2 ± 6.2	4.6 ± 2.3	<i>P</i> = 0.026 , <i>n</i> = 16	11%
BMI (kg/m ²)	30.6 ± 6.4	25.5 ± 4.6	<i>P</i> < 0.001 , <i>n</i> = 16	61%
SAT (cm ³)	6065 ± 3556	3782 ± 2364	<i>P</i> < 0.001 , <i>n</i> = 16	75%
VAT (cm ³)	2112 ± 1430	1166 ± 1128	<i>P</i> < 0.001 , <i>n</i> = 16	62%
HCL (%)	6.6 ± 7.5	2.2 ± 3.6	<i>P</i> = 0.004 , <i>n</i> = 16	62%
SSAT unsaturation (%)	15.2 ± 1.0	14.4 ± 1.5	<i>P</i> = 0.024 , <i>n</i> = 10	51%
IMCL (ratio to Cr)	3.64 ± 1.74	3.64 ± 2.39	<i>P</i> = 0.998, <i>n</i> = 16	2%
DSAT unsaturation (%)	11.4 ± 0.8	11.0 ± 1.0	<i>P</i> = 0.267, <i>n</i> = 16	5%

Data are shown as mean ± SD along with the *P*-value of the paired t-test and the intra-pair resemblance (IPR). *P*-values indicating significant differences and intra-pair correlation coefficients indicating over 50% IPR are in bold. *n* = number of participants, BMI = body mass index, IMCL = intramyocellular lipids, HCL = hepatocellular lipids, SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue, DSAT = deep subcutaneous adipose tissue at the level of the umbilicus, SSAT = superficial subcutaneous adipose tissue from the lower limb. All bolded *P*-values remained significant after controlling for false discovery rate.

P = 0.008) (Table 2), which attenuated when adjusting for sex (*R* = 0.390, *P* = 0.088). The intra-pair difference ΔSSAT associated with ΔBMI (*R* = −0.782, *P* = 0.007) (Table 3), but also attenuated when adjusting for sex (*R* = −0.614, *P* = 0.079). Although male and female twins had similar BMI (27.8 ± 3.2 vs. 28.3 ± 6.2 kg/m²), the males showed higher VAT (2284 ± 1050 vs. 994 ± 651 cm³, *P* = 0.005), HCL (7.3 ± 6.0 vs. 1.48 ± 1.47%, *P* = 0.006) and lower SSAT unsaturation (14.0 ± 0.7 vs. 15.6 ± 0.8%, *P* = 0.004) compared to females, while no sex difference was observed for SAT volume (4080 ± 1694 vs. 5767 ± 2974 cm³, *P* = 0.14), DSAT unsaturation (11.2 ± 0.6 vs. 11.2 ± 0.7%, *P* = 0.96), or IMCL content (3.8 ± 1.7 vs. 3.5 ± 1.4/Cr, *P* = 0.63).

There was no difference in the IMCL content or DSAT unsaturation between heavy and lean twins (Table 1). A strong intra-pair resemblance (*R*² > 0.5, *P* < 0.001), indicating

over 50% explained variance, was observed for BMI, HCL, VAT, SAT, SSAT unsaturation but not for IMCL and DSAT unsaturation (*R*² < 0.03, *P* > 0.2). In the pooled twin data of individual twins, VAT, and SAT correlated positively with BMI, whereas IMCL or DSAT unsaturation did not correlate with BMI (*R*² < 0.04, *P* > 0.2) (Table 2). However, the raw (*R* = −0.462, *P* = 0.001) and sex-adjusted (*R* = −0.463, *P* = 0.001) DSAT unsaturation correlated inversely with IMCL content (Table 2). In line with this, the ΔDSAT associated with ΔIMCL without (*R* = −0.703, *P* = 0.002) or with sex adjustment (*R* = −0.625, *P* = 0.013) (Table 3). The scatterplots between DSAT unsaturation and IMCL are shown in Fig. 1A and B for all twins and for the twin-twin difference.

The lower limb SSAT unsaturation was higher than that of abdominal DSAT (*P* < 0.0001).

Table 2 – Cluster-corrected Pearson correlations coefficients of adipose tissue unsaturation and parameters of body fat distribution in the pooled twin cohort of individual twins.

		SSAT UI	BMI	SAT	VAT	HCL	IMCL
DSAT UI	<i>R</i>	−0.061	−0.145	−0.107	0.265	−0.098	−0.462
	<i>P</i>	0.793	0.321	0.504	0.095	0.623	0.001
	<i>n</i>	20	32	32	32	32	32
SSAT UI	<i>R</i>	–	0.4404	0.549	−0.019	−0.051	−0.179
	<i>P</i>		0.060	0.008	0.921	0.791	0.499
	<i>n</i>		20	20	20	20	20
BMI	<i>R</i>		–	0.879	0.479	0.356	−0.010
	<i>P</i>			≤0.001	0.013	0.088	0.563
	<i>n</i>			32	32	32	32
SAT	<i>R</i>			–	0.380	0.144	0.116
	<i>P</i>				0.031	0.464	0.553
	<i>n</i>				32	32	32
VAT	<i>R</i>				–	0.582	−0.038
	<i>P</i>					0.018	0.786
	<i>n</i>					32	32
HCL	<i>R</i>					–	0.277
	<i>P</i>						0.114
	<i>n</i>						32

R = correlation coefficient (raw), *P* = *P*-value, *n* = number of participants, DSAT UI = unsaturation index of deep subcutaneous adipose tissue, SSAT UI = unsaturation index of superficial subcutaneous adipose tissue, BMI = body mass index, SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue, HCL = hepatocellular lipids, IMCL = intramyocellular lipids. *P*-values indicating significant correlation are in bold. *P*-values that remained significant after controlling for false discovery are underlined.

Table 3 – Spearman rank correlation coefficients of parameters in Table 2 expressed as the difference between twin pairs (Δ = heavy twin – lean twin).

		Δ SSAT UI	Δ BMI	Δ SAT	Δ VAT	Δ HCL	Δ IMCL
Δ DSAT UI	R	–0.067	–0.024	–0.188	0.174	0.012	–0.703
	P	0.855	0.931	0.485	0.520	0.966	0.002
	n	10	16	16	16	16	16
Δ SSAT UI	R	–	–0.782	–0.588	–0.370	–0.152	–0.152
	P	–	0.008	0.074	0.293	0.676	0.676
	n	–	10	10	10	10	10
Δ BMI	R	–	–	0.888	0.735	0.435	–0.044
	P	–	–	<0.001	0.001	0.092	0.871
	n	–	–	16	16	16	16
Δ SAT	R	–	–	–	0.676	0.376	–0.106
	P	–	–	–	0.004	0.151	0.696
	n	–	–	–	16	16	16
Δ VAT	R	–	–	–	–	0.753	–0.118
	P	–	–	–	–	0.001	0.664
	n	–	–	–	–	16	16
Δ HCL	R	–	–	–	–	–	0.006
	P	–	–	–	–	–	0.983
	n	–	–	–	–	–	16

For further details, see Table 2. All bolded P-values remained significant after controlling for false discovery rate.

3.2. DSAT Unsaturation and IMCL in the Second Study

There was also an inverse association between DSAT unsaturation (range 11.0–14.3%) and IMCL (range 2.0–8.9) in the participants of the second cohort ($R = -0.641$, $P = 0.025$), which did not change when adjusting for age or BMI. The repeated sampling at random of one person showed intra-individual day-to-day variability of the IMCL (range 3.0–7.3) and DSAT unsaturation (11.7–15.1%) and an inverse association between DSAT unsaturation and IMCL ($R = -0.765$, $P = 0.027$). Scatterplots for the DSAT unsaturation and IMCL are shown in Fig. 1C.

4. Discussion

In accordance with previous biopsy studies, lower limb SSAT was higher in heavy MZ twins compared to their lean co-twins. In contrast, unsaturation in abdominal DSAT did not differ

between heavy and lean twins and associated inversely with lower limb IMCL content. The inverse association between DSAT unsaturation and IMCL content was further confirmed in healthy volunteers drawn from the population. These results highlight the distinct properties of different adipose depots and their FA composition.

As our initial observation was made in a selected sample of MZ twins discordant for BMI derived from a large population-based twin cohort, twin-to-twin differences can be attributed to external factors that differ between the co-twins, including the proximal contributors to energy imbalance, i.e. energy expenditure as from physical activity and energy intake from diet. Indeed, the heavier twins had higher HCL, VAT and SAT, and higher fasting insulin levels but normal fasting glucose. Also the unsaturation in the lower limb SSAT was higher in the heavy twins, which is in line with a recent observation in adipose tissue biopsies of another set of MZ twins discordant for BMI, where long chain polyunsaturated FA (PUFA) (20:4n-6, 20:5n-3, 22:5n-3) were higher in the abdominal subcutaneous

Fig. 1 – Scatter plots of intramyocellular lipids (IMCL) versus deep subcutaneous adipose tissue (DSAT) unsaturation in (A) all twins combined, for (B) twin intra-pair difference (Δ = heavy twin – lean twin), and (C) in the second cohort (black dots) and for random sampling of one person (gray squares).

adipose tissue of the heavier twin [21]. However, this previous study did not differentiate between the SSAT and DSAT depots. It is known that the unsaturation in deep seated adipose sites is lower than in peripheral [1], a result also reproduced here, as lower limb SSAT was more unsaturated than abdominal DSAT.

Despite the body weight difference, HCL, VAT, SAT, and SSAT unsaturation in the twins still showed strong intra-pair resemblance (IPR). However, neither IMCL nor DSAT unsaturation differed between the discordant co-twins and also showed no IPR. For IMCL content, the lack of IPR is to be expected, as IMCL levels show significant intraday variation due to both physical activity and dietary intake [11,27], factors that are likely to differ in adult free living humans. Similar to this, the twins did not show significant IPR for fasting glucose or insulin. The lack of a difference for DSAT unsaturation between heavy vs. lean twins and the lack of IPR highlight the possibility that DSAT unsaturation displays, like the IMCL depot, a relatively fast response to environmental factors.

In the pooled cohort of individual twins, BMI was strongly associated with SAT and VAT. In line with the higher SSAT unsaturation in the heavier twins, SSAT unsaturation was associated with SAT volume but not with VAT or HCL. In contrast, DSAT unsaturation and IMCL did not associate with BMI, SAT, VAT or HCL, and only showed a strong inverse association to each other. These associations were also present when expressed as twin differences, i.e. heavy-lean twin. Furthermore, the inverse association between DSAT unsaturation and IMCL was also observed in a separate cohort and for the repeated random sampling of one volunteer.

One possible explanation for the inverse association between DSAT unsaturation and IMCL content is the lipolytic activity of adipose tissue, which selectively mobilizes unsaturated FA [3]. In healthy volunteers, IMCL content is positively correlated to VO_2max [28], with already short-term increases in systemic NEFA levels leading to increased IMCL levels [29–33]. Also, insulin resistant humans can display increased IMCL content [12], likely due to impaired suppression of adipose tissue lipolysis by insulin. Accordingly, as adipose tissue is the major source of systemic NEFA in the blood [34], the lipolytic activity of adipose tissue also regulates IMCL levels. As the mobilization rate of a FA increases with FAT unsaturation for any given chain length [3], conditions of higher lipolytic activity would therefore result in a decrease in

adipocyte FA unsaturation. Thus, the association between DSAT unsaturation and IMCL suggests that adipose tissue lipolytic activity decreases the proportion of unsaturated FAs in adipose tissue, while the increased NEFA delivery to skeletal muscle results in higher IMCL levels, as shown in Fig. 2.

The advantage of the present method, ^1H -MRS, resides in the assessment of an unsaturation index from the olefinic and methylene resonance, to which all FAs, including short-chain saturated FA and long-chain PUFA, contribute, even at low concentrations. This advantage over the traditional method of gas chromatography is at same time the main limitation, as no information is provided on individual FAs. As the main hypothesis for selective release of NEFA from adipose tissue suggests that selectivity is based on the degree of unsaturation [3], our unsaturation index is, however, the most sensitive method to probe changes induced by lipolytic activity. Another limitation is that the twin cohort consisted of a relatively small number of MZ BMI discordant twin pairs, which does not allow for advanced modeling of genetic effects. Furthermore, due to the sample size sex effects could not be determined, and although there were sex differences in VAT, HCL and SSAT unsaturation, this did not seem to extend to the association between DSAT unsaturation and IMCL. Another limitation is that the MRS measurements in the twin cohort were performed in the evening with approximately 4 hours of food restriction. Thus, daytime and short duration of food restriction might have impacted the adipose tissue unsaturation and IMCL content. However, the second cohort, measured in the morning after an overnight fast, showed the same association between DSAT unsaturation and IMCL. Also, when adjusting for the time of day of the measurement in the twins, the association between IMCL content and DSAT unsaturation strengthened (data not shown) suggesting these two fat depots are indeed co-regulated.

5. Conclusions

DSAT and SSAT FA unsaturation shows distinct associations with obesity and IMCL in MZ twins reflecting compartment specific metabolic activity. The FA unsaturation in the DSAT depot associates inversely with IMCL content, which suggests that the DSAT depot is in cross talk with the rapid turnover IMCL depot.

Author Contribution

Conduction and design of the study: Lundbom J, Bierwagen, Bodis, Szendrödi, Kaprio, Rissanen, Lundbom, Roden, Pietiläinen. Acquisition, analysis or interpretation of data: Lundbom J, Kaprio, Rissanen, Lundbom, Roden, Pietiläinen. Writing and criticism of the manuscript: Lundbom J, Bierwagen, Bodis, Szendrödi, Kaprio, Rissanen, Lundbom, Roden, Pietiläinen.

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Fig. 2 – Illustration of the proposed mechanistic link between deep subcutaneous adipose tissue (DSAT) unsaturation and intramyocellular lipids (IMCL). Increased lipolytic activity selectively releases unsaturated fat as non-esterified fatty acids (NEFA), which leads to decreased unsaturation in adipose tissue. Subsequent uptake of NEFA in skeletal muscle increases IMCL.

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Disclosure Statement

The authors declare no conflict of interest.

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