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Intramyocellular lipid levels are associated with peripheral but not hepatic insulin sensitivity in normal healthy subjects

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1 **Abstract**

2
3 *Aims*

4 Increased levels of intra-myocellular lipid (IMCL) have been shown to be associated with
5 reduced steady state glucose infusion rates during a hyperinsulinaemic euglycaemic clamp
6 (M-value). Our aim was to explore how IMCL levels relate to the insulin-mediated
7 suppression of endogenous glucose production (hepatic S_I) and increase in glucose disposal
8 (peripheral S_I).
9

10 *Methods*

11 11 healthy young adults (7 male, 4 female) aged 21-31 years undertook, in random order, a
12 hyperinsulinaemic euglycaemic clamp combined with stable glucose isotope enrichment to
13 measure peripheral and hepatic S_I , a ^1H -magnetic resonance spectroscopy (^1H -MRS) scan to
14 determine IMCL levels and a dual energy X-ray absorptiometry (DXA) scan to assess body
15 composition.
16

17 *Results*

18 IMCL levels (range: 3.2-10.7) were associated with whole-body fat mass ($r=0.787$, $p=0.004$),
19 fat mass corrected for height ($r=0.822$, $p=0.002$) and % central fat mass ($r=0.694$, $p=0.02$), but
20 were not related to whole-body fat-free mass (FFM) ($r=-0.472$, $p=0.1$). IMCL levels
21 correlated closely with the M-value ($r=-0.727$, $p=0.01$), FFM-corrected peripheral S_I ($r=-$
22 0.644 , $p=0.03$), but were not related to hepatic S_I adjusted for body weight ($r=0.147$, $p=0.7$).
23

24 *Conclusion*

25 Our data suggest that IMCL accumulation may be a sensitive marker for attenuations in
26 peripheral but not hepatic S_I in normal populations. Given the close relation of IMCL levels to
27 whole-body and central abdominal fat mass, relative increases in the flux of lipids from
28 adipose tissue to the intramyocellular compartment may be an integral part of the mechanisms
29 underlying reductions in S_I .
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1 Introduction

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3 Reduced insulin sensitivity (S_I) is considered to play an important role in the development of
4 type 2 diabetes (T2D) as it is a reproducible finding in patients with manifest T2D [1,2] and
5 their non-diabetic offspring [3]. Furthermore, it has been shown to serve as a predictive
6 marker of the future development of T2D [2,3], which in some cases can be prevented by
7 insulin-sensitising agents [4,5].

8 Twenty years ago, Falholt et al. recognised the intimate relation of increases in muscle fat
9 content to decreases in S_I [6]. Proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) has since
10 provided a non-invasive means of quantifying fat located within myocytes, intra-myocellular
11 lipid (IMCL) [7-9], without exposure to ionising radiation. Numerous studies have shown that
12 IMCL levels are inversely correlated with the M-value (average glucose infusion rate during
13 the steady state of a hyperinsulinaemic euglycaemic clamp) in healthy subjects [10-15] and
14 those with type 1 diabetes (T1D) and T2D [16,17], an association which is demonstrable as
15 early as 11 years of age [11]. However, none of these studies explored how IMCL levels
16 relate to the components that make up whole-body S_I . Insulin has two distinct modes of
17 action: it accelerates glucose disposal, mainly in non-hepatic insulin-dependent tissues, by
18 increasing the number of plasma membrane glucose transporters, whilst also shifting the
19 balance between rates of hepatic glucose storage and release into the circulation in favour of
20 the storage process. Crude models of S_I therefore have two terms, one describing the disposal
21 mechanism, peripheral S_I , and the other expressing the degree of inhibition of endogenous
22 glucose production, hepatic S_I .

23 We studied 11 healthy lean volunteers who had normal glucose tolerance and no family
24 history of diabetes with the aim to define the relation of IMCL levels to peripheral and hepatic
25 S_I , and their association with total and regional body fat mass [11,18-20].

27 Methods

29 *Subjects*

30 Eleven healthy participants (7 male, 4 female) aged 21-31 years were recruited by the
31 University Department of Paediatrics, Addenbrooke's Hospital, Cambridge, UK. The study
32 was approved by the Cambridge Local Research Ethics Committee, and written informed
33 consent was obtained from each volunteer.

34 Participants were known to have normal glucose tolerance according to the 2006 diagnostic
35 criteria of the World Health Organisation, which was determined using an oral glucose
36 tolerance test prior to this study. Furthermore, none of the subjects' parents and siblings had
37 diabetes. As part of this study, subjects undertook a hyperinsulinaemic euglycaemic clamp
38 combined with glucose stable isotope enrichment to measure the rates of endogenous glucose
39 production (R_a) and glucose disposal (R_d), a $^1\text{H-MRS}$ scan to determine IMCL levels and a
40 dual energy X-ray absorptiometry (DXA) scan to assess body composition. In random order,
41 the clamp and DXA scan were conducted on one visit, separated by 7-10 days from the MRS
42 scan. Participants were instructed not to alter their diet and refrain from vigorous exercise 48
43 hours prior to each visit.

45 *$^1\text{H-MRS}$ determination of IMCL*

46 The MRS scan was undertaken at the Wolfson Brain Imaging Centre, Addenbrooke's
47 Hospital. Subjects were placed supine in a Bruker S300 3.0T scanner, with their right leg
48 placed in the centre of the radio frequency coil. The voxel was positioned such that it avoided
49 fasciae planes and visible fat on gradient echo localisation images which were acquired in
50 three orthogonal directions. The voxel was placed in the soleus muscle as opposed to tibialis
51 anterior because of higher IMCL levels and relatively lower variability of extra-myocellular

1 lipid (EMCL) levels in soleus [11,21]. To improve homogeneity of the magnetic field, an
2 automatic and then manual shim were performed. Water suppression was achieved using
3 chemical shift selective (CHESS) and 64 averages of data acquired with an echo time (TE) of
4 35 ms and a repetition time (TR) of 5 seconds using Point Resolved Selective Spectroscopy
5 (PRESS). The original localisation scan was repeated at the end of the experiment to check
6 for any displacement of the leg during the scan.

7 The spectra were analysed in jMRUI [22,23] and fitted using the AMARES algorithm [24]
8 with incorporation of prior knowledge. IMCL levels were quantified by comparing the
9 intensity of the resonance at 1.3 ppm (intracellular triglyceride methylene groups) with that of
10 creatine at 3.0 ppm [8,25].

11 DXA

12 The DXA scan was performed at the Wellcome Trust Clinical Research Facility (WTCRF),
13 Addenbrooke's Hospital, and yielded whole-body fat, central abdominal fat and fat-free mass
14 (FFM). Data on body composition were gathered with a Lunar Prodigy machine using a
15 constant pixel size of 1.2×1.2 cm and Lunar software programmes (version 8.1, Lunar Corp.,
16 Madison, Wisc., USA). The effective radiation dose was 0.2 μ Sievert.

17 *Hyperinsulinaemic euglycaemic clamp*

18 The clamp studies were also carried out at the WTCRF. Subjects were admitted to the ward at
19 07:00 following an overnight fast. The procedure required two intravenous cannulae, one
20 sited in an antecubital vein of each arm. One cannula was used for the infusions of insulin and
21 glucose (20% glucose and glucose isotope) and the other for blood sampling.

22 Three basal blood samples were taken between 07:45 and 08:00. At 08:00, a primed (170 mg)
23 infusion (1.7 mg/min) of [6,6]-²H₂-glucose stable isotope was started and maintained for 5
24 hours to allow determination of glucose turnover. After a stabilisation period of 2.5 hours,
25 blood samples were taken every 5 minutes between 10:30-11:00, and a single-step
26 hyperinsulinaemic euglycaemic clamp was then started with a primed (2.5 mU/kg) infusion
27 (0.5 mU/kg/min) of insulin (11:00-13:00). During the period of hyperinsulinaemia, blood
28 glucose levels were measured every 5 minutes, and maintained as close to 4 mmol/l as
29 possible using a variable rate infusion of 20% glucose solution enriched with 7 mg of [6,6]-
30 ²H₂-glucose per gram of unlabelled glucose in the 20% glucose infusate. At 13:00, all
31 infusions were stopped, cannulae were removed and volunteers were given breakfast.

32 *Glucose isotope composition*

33 ²H isotopic composition was determined by GC/MS (gas chromatography mass spectrometry).
34 For gas chromatographic separation an alkylboronic acid acetate derivative of glucose can be
35 used, customarily α -D-glucofuranose cyclic 1,2:3,5-bis(butylboronate)-6-acetate. We chose to
36 reduce the amount of added hydrogen and increase volatility by replacing butylboronic acid
37 with methylboronic acid and substituting trifluoroacetate for the acetate group. The α -D-
38 glucofuranose cyclic 1,2:3,5-bis(methylboronate)-6-trifluoroacetate derivative was prepared
39 by analogous methods to those used for the conventional analysis [26]. heating at 80°C for 60
40 min. The derivative formed was dried down and reconstituted in 100 μ L 1% trifluoroacetic
41 anhydride in trimethylpentane [27]. Separation was performed by splitless injection onto a 30
42 m DB-1MS column (30 m x 0.25 mm i.d., 0.25 μ m film thickness, Agilent J&W Scientific,
43 Stockport, UK). The injector temperature was 250°C, and the initial column temperature was
44 90°C, held for 1 min, after which it was ramped at 30°C/min to 270°C and maintained at this
45 temperature for a further 0.5 min. Under these conditions the target compound eluted at about
46 4 min. GC/MS analysis of the samples from the clamp studies was performed using a 5973
47 mass selective detector (Agilent Technologies, Stockport, UK). Electron impact ionisation
48 gave an isotopomer cluster in the range m/z 239-243, which corresponds to loss of a 1,2-

1 cyclic-boronate resulting in fragments with the empirical formula $C_7H_8O_5BF_3$. Tracer/tracee
2 ratios were estimated from the relative intensity of the peaks at m/z 242 and 240.

3 4 Assays

5 25 μ l of each blood sample was analysed immediately for glucose levels on a Y.S.I. model
6 2300 stat plus analyser (Lynchford House, Farnborough, UK). The intra-assay coefficient of
7 variation (CV) at 4.1 mM was 1.5%. The equivalent inter-assay CV at this glucose
8 concentration was 2.8%, and 1.7% at 14.1 mM.

9 The remainder of the sample was placed on ice for a maximum of four hours before being
10 separated in a cooled centrifuge, the supernatant drawn off and aliquots frozen at -80°C until
11 analysis. Plasma insulin levels were measured using a DAKO ELISA (DAKO Ltd., Denmark
12 House, Angel Drove, Ely, UK) according to the manufacturer's instructions. The intra-assay
13 CV was 4.3% at 82 pM, 3.0% at 402 pM and 5.7% at 907 pM. The equivalent inter-assay
14 CVs were 4.3%, 5.1% and 5.4% respectively. Plasma non-esterified fatty acid (NEFA) levels
15 were analysed using a WAKO enzymatic colorimetric kit (Alpha Laboratories, Eastleigh,
16 Hampshire) adapted to an ILab 600 clinical chemistry analyser (Instrumentation Laboratories,
17 Warrington, Cheshire). The intra-assay CV was 2.2% at levels of 559 μM and 1143 μM . The
18 equivalent inter-assay CV was 2.5%.

19 20 Calculations

21 BMI was calculated as $[\text{weight (kg)}]/[\text{height (m)}]^2$. DXA scan-derived data were analysed for
22 the whole body and a region representing central fat defined by the top of the pelvis and 8.75
23 cm above, and extending over the full width of the trunk. This provided values for whole-
24 body fat mass, whole-body FFM and % central fat mass. The fat mass index was calculated as
25 $[\text{whole-body fat mass (kg)}]/[\text{height (m)}]^2$, which effectively normalises whole-body fat mass
26 for height [28]. Sex- and age-adjusted standard deviation scores (SDS) for body weight,
27 height and BMI were calculated using the formula $\text{SDS} = (\text{individual's measurement} -$
28 $\text{population mean})/(\text{population SD})$ within the ImsGrowth programme version 2.12 (Medical
29 Research Council, UK).

30 The mean glucose infusion rate during the final 30 min of the clamp (12:30-13:00) provided a
31 crude estimate of peripheral S_i , the M-value (mg/kg/min). Data from the basal pre-clamp and
32 hyperinsulinaemic phases were analysed using Steele's non-steady state equations [29,30],
33 modified for use with stable isotope [31,32]. The effective volume of distribution was
34 assumed to be 146 ml/kg body weight [33]. From the isotope data and average infusion rates
35 of glucose required to maintain euglycaemia, we determined the rates of endogenous glucose
36 production and glucose disposal in the basal (10:30-11:00; R_a^0 and R_d^0 respectively) and
37 hyperinsulinaemic steady states (12:30-13:00; R_a^{SS} and R_d^{SS} respectively). R_a^0 and R_a^{SS} were
38 corrected for kg body weight, and R_d^0 and R_d^{SS} were adjusted for kg FFM. We used the
39 percentage suppression of glucose R_a and percentage increment in glucose R_d from basal to
40 the hyperinsulinaemic steady state, adjusted for the increase in plasma insulin levels, as
41 measures of hepatic and peripheral S_i , respectively.

42 43 Statistics

44 Results are reported as mean (95% confidence interval [CI]) unless stated otherwise.
45 Correlations were tested using the Pearson's correlation coefficient (r). Two-tailed
46 significance was set to $p < 0.05$. Analyses were performed using SPSS for Windows version
47 14.0.

48 49 Results

50
51 Cohort characteristics are summarised by sex in *Table 1*.

1 Results for glucose R_a and R_d , the M-value and levels of IMCL, NEFA, glucose and insulin
2 are listed by sex in *Table 2*. A typical spectra and the corresponding fitting results used to
3 calculate IMCL levels are shown in *Fig. 1*.

4 IMCL levels (range: 3.2-10.7) were associated with whole-body fat mass ($r=0.787$, $p=0.004$),
5 the fat mass index ($r=0.822$, $p=0.002$) and % central fat mass ($r=0.694$, $p=0.02$), but were not
6 related to age ($r=0.338$, $p=0.3$), sex ($r=0.511$, $p=0.1$), weight ($r=0.162$, $p=0.6$), height ($r=-$
7 0.346 , $p=0.3$), BMI ($r=0.496$, $p=0.1$) and whole-body FFM ($r=-0.472$, $p=0.1$). The
8 associations between levels of IMCL and whole-body fat mass ($r=0.773$, $p=0.009$), the fat
9 mass index ($r=0.777$, $p=0.008$) and % central fat mass ($r=0.633$, $p=0.0495$) persisted after
10 adjustment for sex.

11 IMCL levels correlated inversely with the M-value ($r=-0.727$, $p=0.01$). IMCL levels also had
12 an inverse association with peripheral S_I by kg FFM ($r=-0.644$, $p=0.03$; *Fig. 2A*) but were not
13 related to hepatic S_I by kg body weight ($r=0.08$, $p=0.8$; *Fig. 2B*) after adjustment for the
14 increase in plasma insulin levels from baseline to the clamp steady state.

15 Fasting NEFA levels were not associated with IMCL levels ($r=-0.144$, $p=0.7$), peripheral
16 ($r=0.396$, $p=0.3$) or hepatic S_I ($r=-0.004$, $p=0.99$).

18 Discussion

19
20 In this cohort healthy young adults with BMIs <30 kg/m², normal glucose tolerance and no
21 family history of diabetes, we explored the association between ¹H-MRS-determined IMCL
22 levels in the soleus muscle and insulin-stimulated percentage suppression of endogenous
23 glucose production, hepatic S_I , and stimulation of glucose disposal, peripheral S_I , as assessed
24 during a single-step hyperinsulinaemic euglycaemic clamp with [6,6]-²H₂-glucose
25 enrichment. The central finding is a negative correlation between IMCL levels and peripheral
26 S_I , but no relation of IMCL levels to hepatic S_I .

27 Our study is in agreement with previous reports of an association between increases in IMCL
28 levels and reductions in the M-value, a proxy for peripheral S_I , in healthy subjects without a
29 family history of diabetes [10-13]. Three other studies made use of stable glucose isotope
30 during the hyperinsulinaemic euglycaemic clamp while also measuring IMCL levels.
31 However, their methods were limited in the following ways: Perseghin et al. [16] showed that
32 IMCL levels were inversely correlated with the metabolic clearance of glucose in a
33 heterogeneous group of 55 subjects varying up to 30 years in age. The cohort comprised
34 individuals in good health with and without a family history of diabetes as well as subjects
35 who had T1D or T2D. Furthermore, the correlation between IMCL levels and hepatic S_I was
36 not reported. Similarly, Hwang et al. studied 12 subjects, three of whom had a BMI of ≥ 30
37 kg/m², of a wide age range (34 ± 16 years), and did not comment on the associations between
38 levels of IMCL and hepatic S_I [34]. Petersen et al. studied 8 subjects with T2D (mean age: 47
39 years) and 10 lean healthy adults (mean age: 30 years) to show that those with T2D had
40 increased IMCL levels and reduced peripheral and hepatic S_I [17]. However, the authors did
41 not report the results of the analysis of the associations between these measures of S_I and
42 IMCL levels in either subject group.

43 We employed a single low-dose step hyperinsulinaemic euglycaemic clamp with stable
44 glucose isotope enrichment to estimate peripheral and hepatic S_I . The aim was to provide an
45 incomplete suppression of endogenous glucose production during the steady state of the
46 clamp, thereby allowing us to explore potential correlations between the variability in hepatic
47 S_I and IMCL levels, whilst also relating the latter to peripheral S_I during the same dose insulin
48 infusion. Indeed, percentage insulin-mediated suppression of glucose R_a ranged from -126 to -
49 24%, and the change in glucose R_d was between -14 to 292% during the clamp. IMCL levels
50 were not related hepatic S_I corrected for body weight, but had a close inverse association with
51 peripheral S_I after adjustment for FFM. Given the narrow age and BMI range of our cohort,

1 the results suggest that levels of IMCL may be a sensitive and specific marker of the variation
2 in peripheral S_I .

3 The mechanisms by which increases in IMCL levels may lead to reductions in S_I are not
4 precisely understood, and it is worth noting that physical fitness represents a considerable
5 confounding factor when studying these processes [19,35]. Bachmann et al. showed that even
6 a short (6-hour) intravenous infusion of lipid leads to increases in IMCL levels which are
7 mirrored by reductions in the M-value [13]. Thus, muscle triglycerides could directly affect
8 insulin action, or merely be a reservoir of NEFAs which, upon lipolysis, effect decreases in S_I
9 locally that translate into a specific reduction in peripheral S_I [10,36]. NEFAs have been
10 implicated as essential mediators of decreases in S_I : Randle et al. proposed that NEFAs
11 enhance fat oxidation, thereby effecting an accumulation of glucose-6-phosphate in muscle,
12 which in turn inhibits hexokinase II and therefore decreases glucose uptake [37]. Later studies
13 did not substantiate this hypothesis [38-40], and Shulman et al. demonstrated that NEFAs
14 interfere with insulin signalling by reducing phosphatidyl inositol-3 (PI-3) kinase activity
15 [41,42]. Furthermore, insulin receptor autophosphorylation and insulin receptor substrate-1
16 (IRS-1) associated PI-3 kinase activity were found to be reduced in skeletal muscle of
17 individuals with elevated IMCL levels [12]. Alternatively, activation of the hexosamine
18 pathway or various tyrosine phosphatases could be involved in NEFA-induced attenuation of
19 S_I [43,44].

20 We were unable to show an association between fasting circulating levels of NEFA and
21 IMCL, peripheral or hepatic S_I , but our analyses may have been limited by the cohort size.
22 However, given that lipolysis in adipose tissue is far more sensitive to the action of insulin
23 than lipid turnover in muscle [45], intra-myocellular NEFA levels are bound to be highly
24 dependent on the influx of NEFAs from adipocytes [42,46], which are considered to play a
25 crucial role in buffering the circulatory flux of NEFAs in the post-prandial period, in
26 particular [47]. Accordingly, we found close associations between IMCL levels and DXA-
27 derived whole-body fat mass and the fat mass index, even after adjustment for sex, but levels
28 of IMCL were not related to whole-body FFM. Moreover, the distribution of fat across certain
29 body compartments may be more important than the total amount of fat with respect to
30 determining the degree of insulin resistance [48]; central visceral adipose tissue has a higher
31 lipolytic rate than subcutaneous fat, probably because it is less responsive to insulin while
32 being hypersensitive to catecholamines [49-54], and it therefore releases proportionally more
33 NEFAs into the circulation that may then end up being absorbed by myocytes. In our subjects,
34 % central fat mass, as determined by DXA, correlated well with IMCL levels, an association
35 which persisted after adjustment for sex, but the use of DXA did not allow separation of the
36 visceral and subcutaneous compartments of central fat. This may have been of importance in
37 light of Sinha et al.'s report that the inverse correlation between IMCL levels and the M-value
38 is lost once corrected for visceral but not subcutaneous fat [11].

39 In conclusion, our data suggest that, even in normal healthy populations, increases in IMCL
40 levels may be a sensitive marker for attenuations in peripheral but not hepatic S_I .
41 Furthermore, levels of IMCL showed close associations with the total amount of body fat,
42 independent of height, and central abdominal fat mass, suggesting that NEFA "over-supply"
43 from adipose tissue to the intramyocellular compartment may be an integral part of the
44 mechanisms underlying reductions in S_I [42,46].

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47
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1 **Table 1.** Cohort characteristics by sex. Data are means (95% CI).
2

	Male	Female	Combined
Weight (kg)	78.8 74.1-83.5	65.1 37.7-83.5	73.8 65.5-82.1
Height (m)	1.79 1.74-1.84	1.66 1.55-1.77	1.74 1.68-1.80
BMI (kg/m ²)	24.8 22.8-26.7	23.4 16.8-30.0	24.3 22.3-26.2
Whole-body FFM (kg)	59.8 53.4-66.2	40.1 31.0-49.2	52.7 44.8-60.5
Whole-body fat mass (kg)	16.8 8.1-24.1	21.7 4.0-39.5	18.1 11.8-24.5
Fat mass index (kg/m ²)	5.1 2.5-7.7	7.7 2.5-12.9	6.1 4.0-8.1
% central fat mass	27.9 13.9-41.9	38.5 20.0-56.9	31.7 22.1-41.4
Weight SDS	0.75 0.31-1.19	0.51 -2.02-3.04	0.66 0.02-1.30
Height SDS	0.11 -0.61-0.83	0.32 -1.52-2.17	0.18 -0.41-0.78
BMI SDS	0.59 0.00-1.18	0.21 -1.72-2.14	0.45 -0.12-1.02

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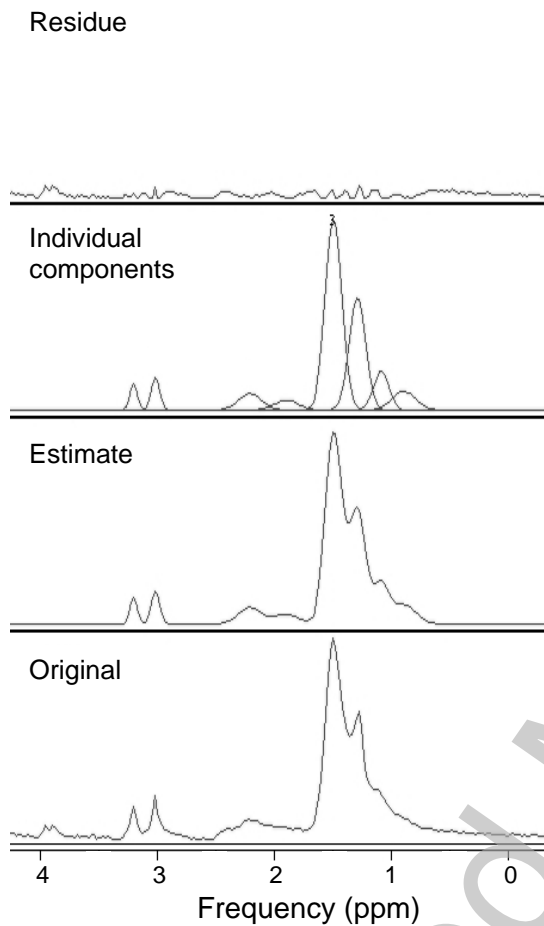
1 **Table 2.** IMCL levels, fasting NEFA levels, levels of glucose and insulin, rates of
 2 endogenous glucose production (R_a) and glucose disposal (R_d) during basal (0) and
 3 hyperinsulinaemic steady states (SS), the M-value, and % change in glucose R_a and R_d from 0
 4 to SS adjusted for the increase in plasma insulin levels from 0 to SS , by sex. Data are means
 5 (95% CI).
 6

	Male	Female	Combined
IMCL (arbitrary units)	5.8 3.8-7.8	8.1 5.0-11.2	6.6 5.1-8.2
Fasting NEFA ($\mu\text{mol/l}$)	523.4 403.7-643.1	328.6 177.8-479.4	436.8 332.1-541.5
Glucose 0 (mmol/l)	4.26 4.10-4.41	4.45 4.14-4.76	4.33 4.20-4.46
Glucose SS (mmol/l)	3.97 3.78-4.15	3.98 3.92-4.04	3.97 3.87-4.08
Insulin 0 (pmol/l)	29.3 27.0-31.6	31.2 25.2-37.1	30.0 28.0-32.0
Insulin SS (pmol/l)	141.3 117.9-164.7	137.1 111.8-162.5	139.8 125.3-154.3
R_a^0 ($\mu\text{mol/min/kg}$)	10.9 9.9-11.8	12.0 10.5-13.4	11.3 10.5-12.0
R_d^0 ($\mu\text{mol/min/FFM}$)	15.3 12.5-18.0	20.0 14.8-25.1	17.0 14.5-19.5
R_a^{SS} ($\mu\text{mol/min/kg}$)	2.1 -1.2-5.3	3.3 -3.2-9.7	2.5 0.1-4.9
R_d^{SS} ($\mu\text{mol/min/FFM}$)	34.4 23.3-45.5	25.0 20.6-29.4	31.0 23.9-38.0
M-value (mg/kg/min)	4.6 3.3-5.9	2.1 0.1-4.1	3.7 2.5-4.9
% change in R_d^{SS-0}	167.5 71.0-264.0	42.5 -23.1-108.2	122.1 51.5-192.6
% change in R_a^{SS-0}	-82.1 -110.0- -54.1	-72.6 -125.5- -19.6	-78.6 -98.8- -58.4

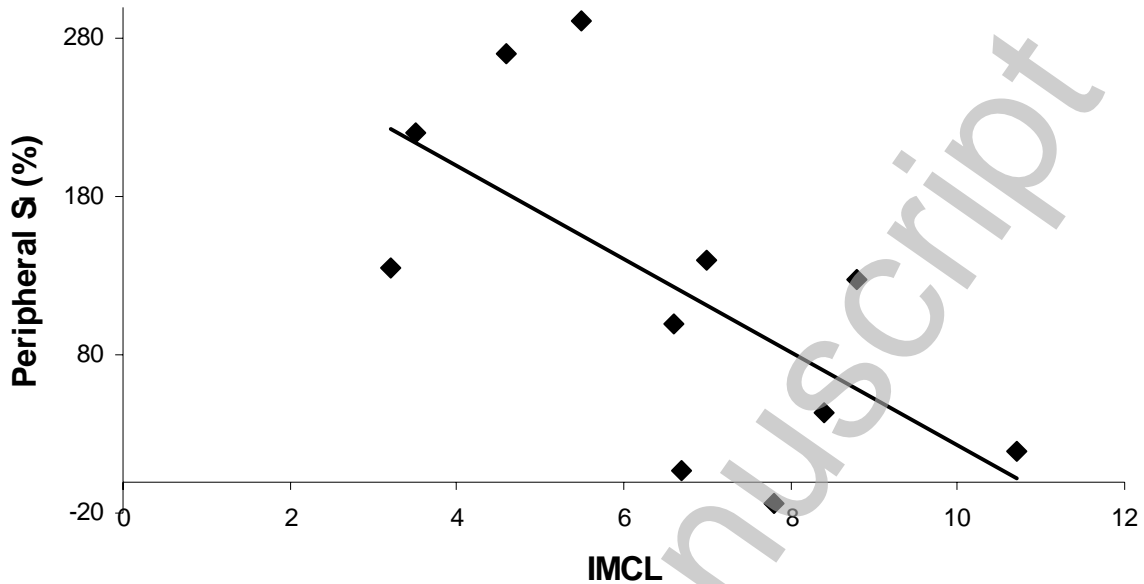
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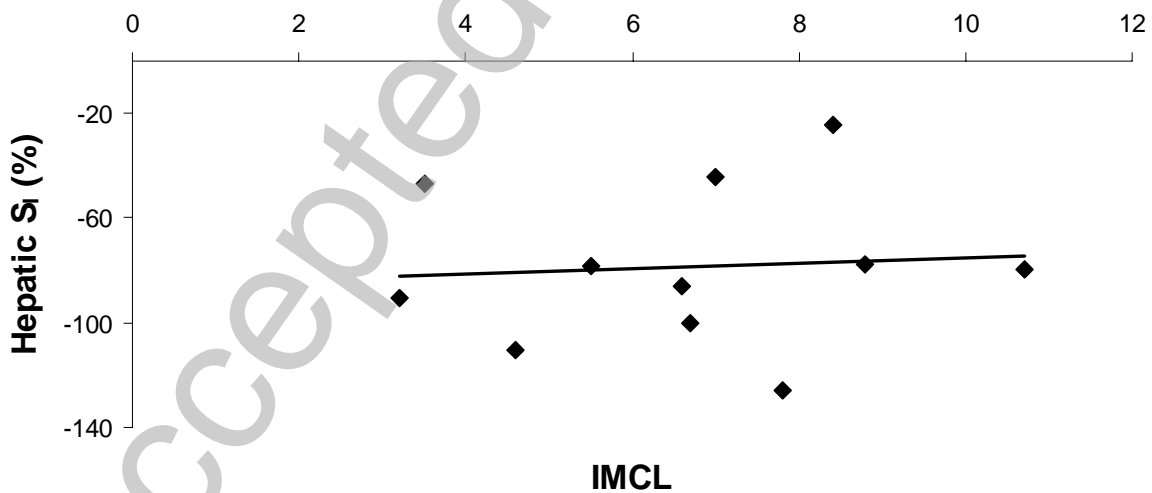
1 **Figure 1.** Representative example of fitted spectra. IMCL (methylene group) found at ~1.3
 2 ppm was quantified relative to the methyl groups of creatine at 3.0 ppm. From bottom
 3 upwards: original spectra, fitted estimate, individual component peaks and residue left after
 4 fitting.



1 **Figure 2A.** Correlation of IMCL levels with peripheral S_I ($r=-0.644$, $p=0.03$), expressed as
 2 the percentage increase in glucose R_d from 0 to SS and corrected for whole-body FFM and
 3 plasma insulin levels.
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 8 **Figure 2B.** Correlation of IMCL levels with hepatic S_I ($r=0.08$, $p=0.8$), expressed as the
 9 percentage decrease in glucose R_a from 0 to SS and corrected for body weight and plasma
 10 insulin levels.
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