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

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ABSTRACT

Increased levels of IMCL (intramyocellular lipid) have been shown to be associated with reduced steady-state glucose infusion rates during a hyperinsulinaemic–euglycaemic clamp (M-value). The aim of the present study was to explore how IMCL levels relate to the insulin-mediated suppression of endogenous glucose production [hepatic SI (insulin sensitivity)] and increase in glucose disposal (peripheral SI). In the present study, 11 healthy young adults (7 male, 4 female; aged 21–31 years) undertook, in random order, an hyperinsulinaemic–euglycaemic clamp combined with stable glucose isotope enrichment to measure peripheral and hepatic SI, a 1H-MRS (proton-magnetic resonance spectroscopy) scan to determine IMCL levels and a DXA (dual-energy X-ray absorptiometry) scan to assess body composition. IMCL levels (range, 3.2–10.7) were associated with whole-body fat mass ($r=0.787, P=0.004$), fat mass corrected for height ($r=0.822, P=0.002$) and percentage of central fat mass ($r=0.694, P=0.02$), but were not related to whole-body FFM (fat-free mass; $r=−0.472, P=0.1$). IMCL levels correlated closely with the M-value ($r=−0.727, P=0.01$) and FFM-corrected peripheral SI ($r=−0.675, P=0.02$), but were not related to hepatic SI adjusted for body weight ($r=0.08, P=0.8$). The results of the present study suggest that IMCL accumulation may be a sensitive marker for attenuations in peripheral, but not hepatic, SI in normal populations. Given the close relationship of IMCL levels to whole-body and central abdominal fat mass, relative increases in the flux of lipids from adipose tissue to the intramyocellular compartment may be an integral part of the mechanisms underlying reductions in SI.

Key words: glucose stable isotope, $^1$H-magnetic resonance spectroscopy, hyperinsulinaemic–euglycaemic clamp, insulin sensitivity, intramyocellular lipid.

Abbreviations: BMI, body mass index; CI, confidence interval; CV, coefficient of variation; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; $^1$H-MRS, proton magnetic resonance spectroscopy; IMCL, intramyocellular lipid; NEFA, non-esterified fatty acid; PI3K, phosphoinositide 3-kinase; $R_a$, endogenous glucose production; $R_a^0$, $R_a$ in the basal state; $R_a^{SS}$, $R_a$ in the hyperinsulinaemic steady state; $R_d$, endogenous glucose disposal; $R_d^0$, $R_d$ in the basal state; $R_d^{SS}$, $R_d$ in the hyperinsulinaemic steady state; SDS, S.D. scores; SI, insulin sensitivity; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; WTCRF, Wellcome Trust Clinical Research Facility.

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INTRODUCTION

Reduced $S_I$ (insulin sensitivity) is considered to play an important role in the development of T2DM (Type 2 diabetes mellitus) as it is a reproducible finding in patients with manifest T2DM [1,2] and their non-diabetic offspring [3]. Furthermore, it has been shown to serve as a predictive marker of the future development of T2DM [2,3], which in some cases can be prevented by insulin-sensitizing agents [4,5].

Over 20 years ago, Falholt et al. [6] recognized the intimate relationship of increases in muscle fat content to decreases in $S_I$ [6]. $^1$H-MRS (proton magnetic resonance spectroscopy) has since provided a non-invasive means of quantifying fat located within myocytes, IMCL (intramyocellular lipid) [7–9], without exposure to ionizing radiation. Numerous studies have shown that IMCL levels are inversely correlated with the M-value (average glucose infusion rate during the steady-state of a hyperinsulinaemic–euglycaemic clamp) in healthy subjects [10–15] and those with T1DM (Type 1 diabetes mellitus) and T2DM [16,17], an association which is demonstrable as early as 11 years of age [11]. However, none of these previous studies explored how IMCL levels relate to the components that make up whole-body $S_I$. Insulin has two distinct modes of action: it accelerates glucose disposal, mainly in non-hepatic insulin-dependent tissues, by increasing the number of plasma membrane glucose transporters, while also shifting the balance between rates of endogenous glucose production (termed $R_S$) and glucose disposal (termed $R_G$). A $^1$H-MRS scan to determine IMCL levels and a DXA (dual-energy X-ray absorptiometry) scan to assess body composition. In a random order, the clamp and DXA scan were conducted on one visit, separated by 7–10 days from the MRS scan. Participants were instructed not to alter their diet and refrain from vigorous exercise 48 h prior to each visit.

$^1$H-MRS determination of IMCL

The MRS scan was undertaken at the Wolfson Brain Imaging Centre, Addenbrooke’s Hospital, Cambridge, U.K. Subjects were placed supine in a Bruker S300 3.0T scanner, with their right leg placed in the centre of the radio frequency coil. The voxel was positioned such that it avoided fasciae planes and visible fat on gradient echo localization images which were acquired in three orthogonal directions. The voxel was placed in the soleus muscle as opposed to tibialis anterior because of higher IMCL levels and relatively lower variability of EMCL (extramyocellular lipid) levels in soleus muscle [11,21]. To improve the homogeneity of the magnetic field, an automatic and then manual shim were performed. Water suppression was achieved using CHESS (chemical shift selective) and 64 averages of data were acquired with a TE (echo time) of 35 ms and a TR (repetition time) of 5 s using PRESS (point resolved selective spectroscopy). The original localization scan was repeated at the end of the experiment to check for any displacement of the leg during the scan. The spectra were analysed in jMRUI ([22] and http://www.mrui.uab.es/mrui/) and fitted using the AMARES algorithm [24] with incorporation of prior knowledge. IMCL levels were quantified by comparing the intensity of the resonance at 1.3 p.p.m. [intracellular triacylglycerol (triglyceride) methylene groups] with that of creatine at 3.0 p.p.m. [8,25].

DXA

The DXA scan was performed at the WTCRF (Wellcome Trust Clinical Research Facility), Addenbrooke’s Hospital, Cambridge, U.K. and yielded whole-body fat, central abdominal fat and FFM (fat-free mass). Data on body composition were gathered with a Lunar Prodigy machine using a constant pixel size of 1.2 cm × 1.2 cm and Lunar software programmes (version 8.1, Lunar). The effective radiation dose was 0.2 μSievert.

Hyperinsulinaemic–euglycaemic clamp

The clamp studies were also carried out at the WTCRF. Subjects were admitted to the ward at 07.00 hours following an overnight fast. The procedure required two intravenous cannulae, one sited in an antecubital vein of each arm. One cannula was used for the infusions of a hyperinsulinaemic–euglycaemic clamp combined with glucose stable isotope enrichment to measure the rates of endogenous glucose production (termed $R_S$) and glucose disposal (termed $R_G$).
insulin and glucose (20% glucose and glucose isotope) and the other for blood sampling.

Three basal blood samples were taken between 07.45 hours and 08.00 hours. At 08.00 hours, a primed (170 mg) infusion (1.7 mg/min) of [6,6]-2H2-glucose stable isotope was started and maintained for 5 h to allow determination of glucose turnover. After a stabilization period of 2.5 h, blood samples were taken every 5 min between 10.30 and 11.00 hours, and a single-step hyperinsulinemic–euglycaemic clamp was then started with a primed (2.5 m-units/kg of body weight) infusion of insulin (11.00–13.00 hours). During the period of hyperinsulinemia, blood glucose levels were measured every 5 min, and maintained as close to 5 mmol/l as possible using a variable rate infusion of 20% glucose solution enriched with 7 mg of [6,6]-2H2-glucose per g of unlabelled glucose in the 20% glucose infusate. At 13.00 hours, all infusions were stopped, cannulae were removed and volunteers were given breakfast.

Glucose isotope composition

The 2H isotopic composition was determined by GC/MS. For gas chromatographic separation an alkylboronic acid acetate derivative of glucose can be used, customarily α-d-glucofuranose cyclic 1,2/3,5-bis(butylboronate)-6-acetate. We chose to reduce the amount of added hydrogen and increase volatility by replacing butylboronic acid with methylboronic acid and substituting trifluoroacetate for the acetate group. The α-d-glucofuranose cyclic 1,2/3,5-bis(methylboronate)-6-trifluoroacetate derivative was prepared by analogous methods to those used for the conventional analysis [26], heating at 80°C for 60 min. The derivative formed was dried down and reconstituted in 100 μl of 1% trifluoroacetic anhydride in trimethylpentane [27].

Separation was performed by splitless injection onto a 30 m DB-1MS column [30 m×0.25 mm i.d. (internal diameter), 0.25 μm film thickness, Agilent J&W Scientific]. The injector temperature was 250°C, and the initial column temperature was 90°C, held for 1 min, after which it was ramped at 30°C/min to 270°C and maintained at this temperature for a further 0.5 min. Under these conditions the target compound eluted at approx. 4 min. GC/MS analysis of the samples from the clamp studies was performed using a 5973 mass selective detector (Agilent Technologies). Electron impact ionization gave an isotopomer cluster in the range m/z 239–243, which corresponds to loss of a 1,2-cyclic-boronate resulting in fragments with the empirical formula C2H4O3BF3. Tracer/tracee ratios were estimated from the relative intensity of the peaks at m/z 242 and 240.

Assays

A 25 μl aliquot of each blood sample was analysed immediately for glucose levels on a YSI model 2300 stat plus analyser. The intra-assay CV (coefficient of variation) at 4.1 mM was 1.5%. The equivalent inter-assay CV at this glucose concentration was 2.8%, and 1.7% at 14.1 mM.

The remainder of the sample was placed on ice for a maximum of 4 h before being separated in a cooled centrifuge, the supernatant drawn off and aliquots frozen at −80°C until analysis. Plasma insulin levels were measured using a DAKO ELISA, according to the manufacturer’s instructions. The intra-assay CV was 4.3% at 82 pM, 3.0% at 402 pM and 5.7% at 907 pM. The equivalent inter-assay CVs were 4.3%, 5.1% and 5.4% respectively. Plasma NEFA (non-esterified fatty acid) levels were analysed using a WAKO enzymatic colorimetric kit (Alpha Laboratories) adapted to an ILab 600 clinical chemistry analyser (Instrumentation Laboratories). The intra-assay CV was 2.2% at levels of 559 μM and 1143 μM. The equivalent inter-assay CV was 2.5%.

Calculations

BMI (body mass index) was calculated as [weight (kg)]/[height (m)]². DXA scan-derived data were analysed for the whole body and a region representing central fat defined by the top of the pelvis and 8.75 cm above, and extending over the full width of the trunk. This provided values for whole-body fat mass, whole-body FFM and percentage central fat mass. The fat mass index was calculated as [whole-body fat mass (kg)]/[height (m)]², which effectively normalizes whole-body fat mass for height [28]. Gender- and age-adjusted SDS (S.D. scores) for body weight, height and BMI were calculated using the formula SDS = (individual’s measurement − population mean)/(population S.D.) within the ImsGrowth programme version 2.12 (Medical Research Council, U.K.).

The mean glucose infusion rate during the final 30 min of the clamp (12.30–13.00 hours) provided a crude estimate of peripheral S1, the M-value (mg·kg⁻¹·min⁻¹ of body weight·min⁻¹). Data from the basal pre-clamp and hyperinsulinemic phases were analysed using Steele’s non-steady-state equations [29,30], modified for use with stable isotope [31,32]. The effective volume of distribution was assumed to be 146 ml/kg of body weight [33]. From the isotope data and average infusion rates of glucose required to maintain euglycaemia, we determined the rates of endogenous glucose production and glucose disposal in the basal (10.30–11.00 hours; Rb and Ra respectively) and hyperinsulinemic steady states (12.30–13.00 hours; Rs and Rs respectively). Rs and Rs were corrected for kg of body weight, and Rs and Rs were adjusted for kg of FFM. We used the percentage suppression of Ra and percentage increment in Rf from basal to the hyperinsulinemic steady state, adjusted for the increase in plasma insulin levels, as measures of hepatic and peripheral S1 respectively.
Cohort characteristics by gender

IMCL levels, fasting NEFA levels, levels of glucose and insulin, rates of

RESULTS

Cohort characteristics are summarized by gender in

Table 1. Results for

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>70.8 (74.1–82.5)</td>
<td>65.1 (37.7–83.5)</td>
<td>73.0 (65.5–82.1)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.79 (1.74–1.84)</td>
<td>1.66 (1.55–1.77)</td>
<td>1.74 (1.68–1.80)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 (22.8–26.7)</td>
<td>23.4 (16.8–30.0)</td>
<td>24.3 (22.3–26.2)</td>
</tr>
<tr>
<td>Whole-body FFM (kg)</td>
<td>59.8 (53.4–64.2)</td>
<td>40.1 (31.0–49.2)</td>
<td>52.7 (44.8–40.5)</td>
</tr>
<tr>
<td>Whole-body fat mass (kg)</td>
<td>16.8 (8.1–24.1)</td>
<td>21.7 (4.0–39.5)</td>
<td>18.1 (11.8–24.5)</td>
</tr>
<tr>
<td>Fat mass index (kg/m²)</td>
<td>5.1 (2.5–7.7)</td>
<td>7.7 (2.5–12.9)</td>
<td>6.1 (4.0–8.1)</td>
</tr>
<tr>
<td>Percentage central fat mass</td>
<td>27.9 (13.9–41.9)</td>
<td>38.5 (20.0–56.9)</td>
<td>31.7 (22.1–41.4)</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.75 (0.31–1.19)</td>
<td>0.51 (−2.02–3.04)</td>
<td>0.66 (0.02–1.30)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.11 (−0.61–0.83)</td>
<td>0.32 (−1.52–2.17)</td>
<td>0.18 (−0.41–0.78)</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.59 (0.00–1.18)</td>
<td>0.21 (−1.72–2.14)</td>
<td>0.45 (−0.12–1.02)</td>
</tr>
</tbody>
</table>

Table 2 IMCL levels, fasting NEFA levels, levels of glucose and insulin, rates of Rₐ and R₆ during basal (0) and hyperinsulinaemic steady states (55), the M-value, and percentage change in Rₐ and R₆ from 0 to 55 adjusted for the increase in plasma insulin levels from 0 to 55, by gender

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMCL (arbitrary units)</td>
<td>5.8 (3.8–7.8)</td>
<td>8.1 (5.0–11.2)</td>
<td>6.6 (5.1–8.2)</td>
</tr>
<tr>
<td>Fasting NEFA (µmol/l)</td>
<td>521.4 (403.7–643.1)</td>
<td>328.6 (177.8–479.4)</td>
<td>436.8 (332.1–541.5)</td>
</tr>
<tr>
<td>Glucose a (mmol/l)</td>
<td>4.26 (4.10–4.41)</td>
<td>4.45 (4.14–4.76)</td>
<td>4.33 (4.20–4.46)</td>
</tr>
<tr>
<td>Glucose b (mmol/l)</td>
<td>3.97 (3.78–4.15)</td>
<td>3.98 (3.92–4.04)</td>
<td>3.97 (3.87–4.08)</td>
</tr>
<tr>
<td>Insulin a (pmol/l)</td>
<td>29.3 (27.0–31.6)</td>
<td>31.2 (25.2–37.1)</td>
<td>30.0 (28.0–32.0)</td>
</tr>
<tr>
<td>Insulin b (pmol/l)</td>
<td>141.3 (117.9–164.7)</td>
<td>137.1 (111.8–162.5)</td>
<td>139.8 (125.3–154.3)</td>
</tr>
<tr>
<td>Rₘ a (µmol·min⁻¹·kg⁻¹ of body weight)</td>
<td>10.9 (9.9–11.8)</td>
<td>12.0 (10.5–13.4)</td>
<td>11.3 (10.5–12.0)</td>
</tr>
<tr>
<td>Rₘ b (µmol·min⁻¹·FFM⁻¹)</td>
<td>15.3 (12.5–18.0)</td>
<td>20.0 (14.8–25.1)</td>
<td>17.0 (14.5–19.5)</td>
</tr>
<tr>
<td>R₄ a (µmol·min⁻¹·kg⁻¹ of body weight)</td>
<td>2.1 (1.2–5.3)</td>
<td>3.3 (3.2–9.7)</td>
<td>2.5 (0.1–4.9)</td>
</tr>
<tr>
<td>R₄ b (µmol·min⁻¹·FFM⁻¹)</td>
<td>34.4 (23.3–45.5)</td>
<td>25.0 (20.6–29.4)</td>
<td>31.0 (23.9–38.0)</td>
</tr>
<tr>
<td>M-value (mg·min⁻¹·kg⁻¹ of body weight)</td>
<td>4.6 (3.3–5.9)</td>
<td>2.1 (0.1–4.1)</td>
<td>3.2 (2.5–4.9)</td>
</tr>
<tr>
<td>Percentage change in Rₘ a</td>
<td>130.5 (49.5–211.6)</td>
<td>28.1 (16.8–73.1)</td>
<td>93.3 (25.1–151.5)</td>
</tr>
<tr>
<td>Percentage change in Rₘ b</td>
<td>−82.1 (−110.1–−54.1)</td>
<td>−72.6 (−125.6–−19.6)</td>
<td>−78.6 (−98.8–58.4)</td>
</tr>
</tbody>
</table>

Statistics

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

RESULTS

Cohort characteristics are summarized by gender in Table 1. Results for Rₐ and R₆, the M-value and levels of IMCL, NEFA, glucose and insulin are listed by gender in Table 2. A typical spectra and the corresponding fitting results used to calculate IMCL levels are shown in Figure 1.

IMCL levels (range, 3.2–10.7) were associated with whole-body fat mass (r = 0.787, P = 0.004), the fat mass index (r = 0.822, P = 0.002) and percentage central fat mass (r = 0.694, P = 0.02), but were not related to age (r = 0.338, P = 0.3), gender (r = 0.511, P = 0.1), weight (r = 0.162, P = 0.6), height (r = −0.346, P = 0.3), BMI (r = 0.496, P = 0.1) and whole-body FFM (r = −0.472, P = 0.1). The associations between levels of IMCL and whole-body fat mass (r = 0.773, P = 0.009), the fat mass index (r = 0.777, P = 0.008) and percentage central fat mass (r = 0.633, P = 0.0495) persisted after adjustment for gender.

IMCL levels correlated inversely with the M-value (r = −0.727, P = 0.01). IMCL levels also had an inverse association with peripheral S_i by kg of FFM (r = −0.675, P = 0.02; Figure 2A), but were not related to hepatic S_i by kg of body weight (r = 0.08, P = 0.8; Figure 2B) after adjustment for the increase in plasma insulin levels from baseline to the clamp steady state.

Fasting NEFA levels were not associated with IMCL levels (r = −0.144, P = 0.7), or peripheral (r = 0.396, P = 0.3) or hepatic S_i (r = −0.004, P = 0.99).
DISCUSSION

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

The present study is in agreement with previous reports of an association between increases in IMCL levels and reductions in the M-value, a proxy for peripheral SI, in healthy subjects without a family history of diabetes [10–13]. Three other studies made use of stable glucose isotope during a single-step hyperinsulinaemic–euglycaemic clamp with [6,6]-2H₂-glucose enrichment. The central finding is a negative correlation between IMCL levels and peripheral SI, but no relation of IMCL levels to hepatic SI.

In the present study, we employed a single low-dose step hyperinsulinaemic–euglycaemic clamp with stable glucose isotope enrichment to estimate peripheral and hepatic SI. The aim was to provide an incomplete suppression of endogenous glucose production during the steady-state of the clamp, thereby allowing us to explore potential correlations between the variability in hepatic SI and IMCL levels, while also relating the latter to peripheral SI during the same dose insulin infusion. Indeed, percentage insulin-mediated suppression of RA ranged from −126 to −24 %, and the change in RA was
from −14 to 292 % during the clamp. IMCL levels were not related to hepatic SI corrected for body weight, but had a close inverse association with peripheral SI after adjustment for FFMI. Given the narrow age and BMIR of the cohort used in the present study, the results suggest that levels of IMCL may be a sensitive and specific marker of the variation in peripheral SI.

The mechanisms by which increases in IMCL levels may lead to reductions in SI are not precisely understood, and it is worth noting that physical fitness represents a considerable confounding factor when studying these processes [19,35]. Bachmann et al. [13] showed that even a short (6 h) intravenous infusion of lipid leads to increases in IMCL levels which are mirrored by reductions in the M-value [13]. Thus muscle triacylglycerols could directly affect insulin action, or merely be a reservoir of NEFAs which, upon lipolysis, effect decreases in SI locally that affect insulin action, or merely be a reservoir of NEFAs in IMCL levels which are mirrored by reductions in the short (6 h) intravenous infusion of lipid leads to increases in peripheral SI. Randle et al. [37] proposed that NEFAs may lead to reductions in SI are not precisely understood, may be an integral part of the mechanisms underlying reductions in SI [42,46].

In conclusion, the results of the present study suggest that, even in normal healthy populations, increases in IMCL levels may be a sensitive marker for attenuations in peripheral, but not hepatic, SI. Furthermore, levels of IMCL showed close associations with the total amount of body fat, independent of height, and central abdominal fat mass, suggesting that NEFA ‘over-supply’ from adipose tissue to the intramyocellular compartment may be an integral part of the mechanisms underlying attenuations of IMCL [43,44].

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