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Rapid communication

Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study

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Summary Recent muscle biopsy studies have shown a relation between intramuscular lipid content and insulin resistance. The aim of this study was to test this relation in humans by using a novel proton nuclear magnetic resonance (^{1}H NMR) spectroscopy technique, which enables non-invasive and rapid (~ 45 min) determination of intramyocellular lipid (IMCL) content. Normal weight non-diabetic adults (n = 23, age 29 ± 2 years, BMI = 24.1 ± 0.5 kg/m^2) were studied using cross-sectional analysis. Insulin sensitivity was assessed by a 2-h hyperinsulinaemic (~ 450 pmol/l)-euglycaemic (~ 5 mmol/l) clamp test. Intramyocellular lipid concentrations were determined by using localized ^{1}H NMR spectroscopy of soleus muscle. Simple linear regression analysis showed an inverse correlation (r = –0.692, p = 0.0017) between intramyocellular lipid content and M-value (100–120 min of clamp) as well as between fasting plasma non-esterified fatty acid concentration and M-value (r = –0.54, p= 0.0267). Intramyocellular lipid content was not related to BMI, age and fasting plasma concentrations of triglycerides, non-esterified fatty acids, glucose or insulin. These results show that intramyocellular lipid concentration, as assessed non invasively by localized ^{1}H NMR spectroscopy, is a good indicator of whole body insulin sensitivity in non-diabetic, non-obese humans. [Diabetologia (1999) 42: 113–116]

Keywords Intramyocellular lipids, insulin sensitivity, NMR spectroscopy, skeletal muscle, triglycerides.

Skeletal muscle insulin resistance is a common feature of obesity, dyslipidaemia and arterial hypertension, and it is an important predisposing factor for Type II (non-insulin-dependent) diabetes mellitus and premature cardiovascular disease [1]. Evidence has been provided that lipids could have an important role in insulin resistance: i) lipid oxidation is increased in insulin resistant states [2] and ii) increase of plasma concentrations of non-esterified fatty acids (NEFA) decreases skeletal muscle glucose uptake and glycogen synthesis [3]. The impact of intramyocellular lipid (IMCL) content on insulin sensitivity has previously been examined from muscle biopsies. The results showed that IMCL are an important source of energy within the muscle [4] and that increased IMCL content is associated with impaired insulin-stimulated glucose uptake in rats [5] as well as in healthy humans [6, 7] and in those with Type I (insulin-dependent) diabetes mellitus [8].

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy now enables non-invasive quantification of the IMCL content in humans [9–11]. This study was designed: i) to examine the cross-sectional relation between IMCL and whole body insulin sensitivity in non-diabetic humans by using non-invasive localized proton NMR spectroscopy and ii) to compare the relative contributions of IMCL, BMI and

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Abbreviations: NMR, Nuclear magnetic resonance; IMCL, intramyocellular lipids; NEFA, non-esterified fatty acids; TG, triglycerides; M-value, rate of whole-body glucose uptake.

plasma triglyceride (TG) concentration with whole body insulin sensitivity.

Methods

Subjects. We studied 23 (8 men/15 women) normal weight subjects who were in good health and had no family history of diabetes. All subjects gave informed written consent and the studies were approved by the Human Investigation Committee of Yale University School of Medicine, New Haven, Conn., USA.

Hyperinsulinaemic-euglycaemic clamp. On the morning of the study Teflon catheters (Johnson & Johnson Medical Inc., Arlington, Tex., USA) were inserted into antecubital veins in the right arm for drawing blood and in the left arm for infusion of glucose and insulin. Conditions of insulin-stimulated whole-body glucose uptake were achieved by doing hyperinsulinaemic-euglycaemic clamp tests for 120 min [12]. Insulin (Humulin-Regular, Lilly, Indianapolis, Ind., USA) was given as a primed-continuous intravenous infusion (240 pmol $\cdot$ m^{-2} $\cdot$ min^{-1}) resulting in constant hyperinsulinaemia of approximately 450 pmol/l. Plasma glucose concentrations were kept at approximately 5 mmol/l by a variable intravenous glucose infusion (Dextrose 1.1 mol/l). Rates of whole-body glucose uptake (M-value) were calculated as the mean glucose infusion rates from 100 to 120 min, corrected for glucose space and urinary glucose excretion, and expressed in micromols per minute per kilogram body weight.

In vivo ^{1}H-NMR spectroscopy. Localized ^{1}H NMR spectra of the soleus muscle were acquired on a 2.1 T/1 m Biospec system (Bruker Instruments, Inc., Billerica, Mass., USA) by using a 16 cm circular surface coil in a transmitter/receiver mode. During the measurements, the subject remained in supine position within the spectrometer. The gastrocnemius-soleus muscle complex of the right leg was positioned within the homogeneous volume of the magnet on top of the coil. The magnetic field was shimmed on non-localized water signal (usual bandwidth ~ 50 Hz). Scout images were acquired in order to position the volume of interest. Stimulated echo acquisition mode spectroscopic sequence [13] (echo time = 30 ms, repetition time = 2 s, 128 averages, 2048 data points) complemented by spatially localized suppression pulse centred into the adipose tissue layer was used on a volume of [1.5 cm]^3. After line broadening, phase and baseline correction, the intensities of the IMCL resonance were estimated by line fitting the spectra. After T_1 and T_2 relaxation correction, quantification of the IMCL content was done by comparing the intensity of methylene [$(CH_2)_n$-; 1.25 ppm] resonance with that of water. Spectra were processed and the resonances quantified using the MacNuts-PPC Software package (AcornNMR Inc., Calif., USA). The IMCL concentration is expressed as percentage of the intensity of the water resonance peak. The measurements were carried out at least 2–3 h postprandially within a week after the clamp test.

Plasma analysis. Plasma glucose was measured by the glucose oxidase method (Glucose analyzer II, Beckman Instruments, Fullerton, Calif., USA). Plasma insulin was determined by a double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, Tex., USA). Fasting plasma triglyceride concentrations were determined by a commercially available assay (C336–10, Sigma, St. Louis, Mo., USA). Plasma non-esterified fatty acid (NEFA) concentrations were measured by a microfluorimetrical method.

Statistical analysis. All statistical analyses were made using the StatView 4.5 package (Abacus Concepts, Berkeley, Calif., USA). Simple, multiple, and stepwise linear regressions were used to detect correlation among variables. Data are presented as means ± SEM.

Results

Clinical and laboratory characteristics, insulin-stimulated whole-body glucose uptake (M-value) and intramyocellular lipid (IMCL) content of the subjects are summarized in Table 1.

A typical localized proton NMR spectrum of the soleus muscle is shown in Figure 1A. Linear regression analysis (Fig. 1B) of insulin-stimulated whole-body glucose uptake against the IMCL content showed an inverse correlation ($n = 23$, $r = –0.69$, $p = 0.0017$). A further correlation was detected between fasting plasma NEFA and M-value ($r = –0.54$, $p = 0.0267$). No correlation was found between BMI and M-value ($r = 0.11$; $p = 0.748$), fasting plasma TG and M-value ($r = 0.20$; $p = 0.563$), fasting plasma insulin and M-value ($r = –0.34$, $p = 0.163$), fasting plasma glucose and M-value ($r = 0.04$, $p = 0.844$), as well as age and M-value ($r = 0.08$, $p = 0.762$).

Multiple regression of all permutations of independent relations between IMCL content, BMI, age, fasting plasma concentrations of TG, NEFA, insulin, and glucose showed that the correlation between IMCL content and M-value was independent of BMI, fasting plasma glucose and age. Combining IMCL content with either fasting plasma TG or fasting plasma insulin or fasting plasma NEFA concentration in a multiple regression of M-value showed correlation in all cases (TG: $r = –0.822$, $p = 0.006$; insulin: $r = 0.658$, $p = 0.045$; NEFA: $r = –0.714$, $p = 0.0104$). Correlation between NEFA and M-value was also independent of all remaining variables but none of them improved the correlation coefficient. None of the permutations without IMCL content or NEFA concentration showed a correlation with M-value.

In a stepwise regression of all possible predictors (IMCL, BMI, age and fasting plasma concentrations of TG, NEFA, insulin, or glucose) of insulin resistance, first IMCL content and then fasting plasma NEFA concentration were identified as independent correlates, with BMI, age and fasting plasma concentrations of glucose, insulin, and TG not entering the regression. Forcing the age to enter the regression gradually improved the correlation ($r = –0.907$ with age vs $r = –0.877$ without age entering the regression).

Table 1. Clinical, laboratory characteristics, insulin sensitivity (M-value 100–120 min) and intramyocellular lipid (IMCL) content in 23 healthy non-diabetic subjects

	Mean	Range
Age (years)	29 ± 2	19 – 70
BMI (kg/m^2)	24.1 ± 0.5	20.6 – 26.9
Fasting plasma glucose (mmol/l)	4.93 ± 0.06	4.44 – 5.44
Fasting plasma insulin (pmol/l)	51.8 ± 1.9	36 – 66
Fasting plasma triglycerides (mmol/l)	1.17 ± 0.17	0.44 – 3.56
Fasting plasma non-esterified fatty acids (μmol/l)	532.6 ± 41.0	181 – 811
M-value (μmol · kg^{-1} · min^{-1})	36.3 ± 3.7	11.1 – 81.3
IMCL content (% of water resonance peak intensity)	2.21 ± 0.11	1.27 – 3.01

Discussion

It is known that a short-term increase in circulating non-esterified fatty acid concentrations decreases insulin-stimulated whole-body glucose uptake [2] due to reduction of skeletal muscle glucose uptake and glycogen synthesis [3]. Our study points to an important role of the intramyocellular lipid stores as indicators of whole-body insulin sensitivity. It is noteworthy that intramyocellular lipids are immediately available for mitochondrial oxidation and might therefore interfere with intracellular glucose metabolism.

Some, but not all, previous invasive studies which used biopsies from human skeletal muscle suggested a relation between skeletal muscle lipids and insulin sensitivity. A study of older normoglycaemic women found that intracellular lipid content, as assessed histologically by Oil red O staining of muscle fibres was inversely correlated with muscle insulin sensitivity as determined by insulin-stimulated muscle glycogen synthase activity [7]. This measure of insulin sensitivity was correlated in a similar fashion with biochemically determined muscle TG concentrations. Intracellular lipids, were also related to waist-to-hip ratio and fasting plasma NEFA, but not to glucose tolerance or whole-body insulin sensitivity as assessed from an insulin tolerance test. Another study [6] reported an inverse correlation between muscle TG and insulin sensitivity as estimated during euglycaemic and hyperglycaemic clamp tests in insulin resistant (mean M-value of ~ 17.8 μmol · kg^{-1} · min^{-1}) and obese (mean BMI of ~ 32.7 kg · m^{-2}) Pima Indians. Measures of obesity (BMI, percentage of body fat, waist-to-hip ratio) were also inversely correlated with insulin-stimulated whole-body glucose uptake but not with muscle TG concentrations. Multiple linear regression analysis showed that muscle TG predicts insulin sensitivity independently of all of those measures. Using similar techniques, no correlation between muscle TG content and insulin sensitivity could be detected in either lean non-diabetic or Type I diabetic humans [8]. Interestingly, fasting muscle TG concentrations were clearly higher in Type I diabetic humans under conditions of good metabolic control.

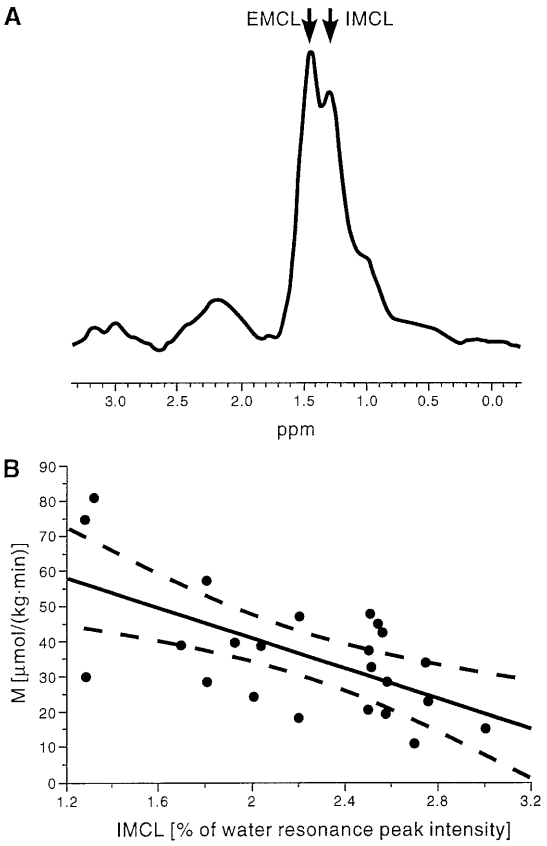

Fig. 1. A, B Figure **A** shows an enlarged region of a typical localized ^{1}H NMR spectra of the soleus muscle. "IMCL" denotes the = $(CH_2)_n$ resonance of the intramyocellular lipids (1.25 ppm) and "EMCL" denotes the = $(CH_2)_n$ resonance (1.4 ppm) of the extramyocellular lipids. Resonances of creatine (3.00 ppm) and choline (3.2 ppm) are resolved as well. **B** Relation between the insulin sensitivity (M-value 100–120 min hyperinsulinaemic-euglycaemic-clamp) and intramyocellular lipid (IMCL) content of 23 healthy non-diabetic subjects. The linear regression line [M = 83.7–21.4 * (IMCL), $n = 23$, $r = -0.692$, $p = 0.0017$] is drawn solid, lines representing the 95 % confidence interval are dashed

This study found a negative correlation between intramyocellular lipid concentrations as measured by localized ^{1}H NMR spectroscopy and the insulin-stimulated whole-body glucose uptake. We have confirmed that this relation is not only true for insulin resistant and obese groups [6] but even for non-obese subjects with normal insulin sensitivity [7]. Similar results have been recently presented as an abstract [14]. In addition, our study found that skeletal muscle insulin sensitivity is primarily affected by IMCL stores and secondarily by circulating plasma NEFA concentrations. Intramyocellular lipid stores as measured by ^{1}H NMR spectroscopy might therefore be an excellent predictor of insulin sensitivity in non-diabetic

subjects. The correlation observed between fasting plasma NEFA concentration and insulin-stimulated whole-body glucose uptake is in good agreement with the short-term effects of an increase in plasma NEFA concentration on muscle insulin sensitivity [3]. The failure to detect a correlation between BMI and insulin-stimulated whole-body glucose uptake as well as between BMI and IMCL content could be due to the narrow range of BMI in the group studied. We found, however, that age made only a minor contribution to the M-value, despite its broad range in the studied group. This can be explained by the non-equal distribution of age in our subjects, as only three subjects were over 40 years old.

It is noteworthy that direct comparison of the available studies is limited because different populations and techniques have been used for the assessment of intramyocellular lipids or muscle triglycerides. The IMCL content as measured by our method is expressed in relative units. Determination of the amount of methylene groups in the individual fatty acid chains does not take account of the distribution of different lengths of fatty acid chains. The degree of lipid saturation also cannot be accessed by this measurement. Although different lipid resonances can be theoretically quantified by ^{1}H NMR spectroscopy, which would enable detailed insight into skeletal muscle lipid composition, other lipid resonances are not clearly resolved under most in vivo conditions. Thus, future development of NMR techniques towards the use of stronger and more homogeneous magnetic fields is required to improve this approach.

In summary, we found a negative correlation between intramyocellular lipid content and whole-body insulin sensitivity, which accords with previous findings from muscle biopsy studies in rats and humans. The method presented is non-invasive and enables measurement of the lipid content in different muscle groups separately within 45 min.

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