Magnetic Resonance in Medicine 45:179–183 (2001)

Fast Elevation of the Intramyocellular Lipid Content in the Presence of Circulating Free Fatty Acids and Hyperinsulinemia: A Dynamic ^{1}H-MRS Study

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The influence of a short-term elevation of free fatty acids (FFAs) on intramyocellular lipids (IMCL) under hyperinsulinemic conditions was monitored in five healthy male subjects in the course of a 5-hr hyperinsulinemic glucose clamp. During the glucose clamp a lipid emulsion (Intralipid 20®) and heparin were administered intravenously. IMCL was quantified in the tibialis anterior (TA) and the soleus (SOL) muscle by ^{1}H-MRS. A rapid elevation of the IMCL pool was found in both muscles (61% in TA and 22% in SOL) in the 5-hr time period. A control hyperinsulinemic glucose clamp in the same study group, repeated without elevation of circulating FFAs, did not lead to significant changes in IMCL for both muscles. The present study shows for the first time that only the combination of high concentrations of FFAs and insulin lead to marked storage of lipids in skeletal muscle cells in humans. Magn Reson Med 45:179–183, 2001.

Key words: 1H-MRS; muscular lipids, IMCL; diabetes; hyperinsulinemia

Skeletal muscle insulin resistance of glucose uptake is a disorder frequently seen in different metabolic diseases, such as type 2 diabetes, obesity, dyslipidemia, and arterial hypertension (1,2). There is evidence that regulation of lipid metabolism plays an important role in insulin resistance (3,4). In insulin-resistant states, lipid oxidation is increased (5). Furthermore, the experimental elevation of circulating free fatty acids (FFAs) was found to augment lipid oxidation and to decrease skeletal muscle glucose uptake and glycogen synthesis (6,7). Moreover, in animal and clinical studies, insulin resistance can be induced by fat feeding (8–10). An increased fat intake was associated with an augmented lipid content in the skeletal muscle, and the latter was found to be closely correlated to the degree of insulin resistance (8,11,12).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is well established for the noninvasive quantification of muscular lipids in vivo. Due to the geometric arrangement of fat within the muscle cell (intramyocellular lipids [IMCL]) and extracellular adipocytes (extramyocellular lipids [EMCL]), ^{1}H-MRS is able to distinguish between these lipid compartments (13–15). Using ^{1}H-MRS, several groups recently reported a negative correlation between IMCL of skeletal muscle and insulin sensitivity of glucose uptake (16–20).

However, the regulatory factors on IMCL are only partly known. While clinical studies indicate that IMCL decreases within hours after moderate prolonged exercise (21,22), little is known about the formation of IMCL. Only one animal study showed that total skeletal muscle lipids can be rapidly increased by infusion of lipid emulsion and heparin in the presence of hyperinsulinemia (23). On the other hand, Krssak et al. (24) did not find any influence of elevated circulating FFAs on IMCL under euglycemic and normoinsulinemic conditions in humans.

The aim of the present study was to find out whether an increase of circulating FFAs and prolonged experimental hyperinsulinemia induce rapid changes of IMCL in humans, and whether the time course can be followed by ^{1}H-MRS.

METHODS

Subjects

Five male Caucasian subjects, 24–34 years old (mean age ± SD: 29.2 ± 3.8 years), were studied. The subjects were not taking any medication. Body mass indices (BMIs) and waist-to-hip ratios (WHRs) of all subjects were in a normal range (mean BMI: 21.7 kg/m^2, range: 18.7–23.3 kg/m^2; mean WHR: 0.86, range: 0.8–1.0). All participants had a normal oral glucose tolerance test according to the World Health Organization criteria (fasting blood glucose level <100 mg/dl, and 2 hr after the intake of 75 g glucose <120 mg/dl; WHO, 1990). All individuals gave informed, written consent, and the studies were approved by the local ethics committee of the University of Tuebingen.

Study Design

After an overnight fast, the volunteers reported to the MR unit. Teflon catheters (Braun, Germany) were inserted into antecubital veins in the right arm for taking blood and in the left arm for infusion. The subjects were positioned and remained in the magnet throughout the whole study. A hyperinsulinemic euglycemic glucose clamp was performed for 5 hr. Constant hyperinsulinemia was achieved by a continuous intravenous insulin infusion (Insuman®, Hoechst, Ger-

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Received 4 August 2000; revised 6 September 2000; accepted 29 September 2000.

many) of 1.0 mU/kg/min. Plasma glucose was measured every 5 min by the glucose-oxidase method (Yellow Springs Instruments, Ohio), and was kept in the euglycemic range by a variable intravenous glucose infusion (40%). Blood was taken every 20 min for the determination of serum insulin (MEIA Abbott, Wiesbaden, Germany).

Plasma FFAs were elevated via infusion of a lipid emulsion and heparin, and measured every 20 min. After a bolus of 250 IU, heparin (10000 I.E./ml; Heparin-Natrium Braun "Multi"; Braun, Germany) and 20% lipid emulsion (Intralipid 20®, Pharmacia & Upjohn) were infused with a constant rate of 0.4 IU/kg/min and 1.5 ml/min, respectively.

Control Group

The same five subjects served as the control group. To exclude effects of hyperinsulinemia on IMCL per se, the study group was examined again. The conditions were chosen as before, but instead of the infusion of Intralipid 20® and heparin 0.9%, isotonic NaCl solution (NaCl 0.9%; Delta-Pharma, Germany) was infused in the antecubital vein for 5 hr with an identical constant flow rate of 1.5 ml/min. Plasma glucose and insulin levels were controlled as described above.

In Vivo ^{1}H-MRS

Two muscles of the lower leg were examined: the tibialis anterior muscle (TA), representing a muscle of mixed type I and II fibers; and the soleus muscle (SOL), representing a muscle of predominantly type I fibers with high oxidative capacity. The subjects underwent MRI and spectroscopy parallel to the metabolic examinations, resting in the magnet in a supine position for the entire 5-hr time period. ^{1}H-MRS was performed on a 1.5 Tesla whole-body system (Magnetom Vision; Siemens, Erlangen, Germany). Image-guided localized proton spectra with a voxel size of 2.4 cm^3 were recorded from representative regions in the muscles using the circular polarized standard extremity coil of the manufacturer. The leg was fixed with a cast to prevent motion artifacts and position changes during the measurements. Spectra were recorded every 60 min, including a baseline spectrum at t = 0, i.e., before any of the described substances were infused. Volume-selective shimming was performed for each new voxel position. Spectra were recorded by a STEAM technique (TR = 2 sec, TE = 10 msec, and TM = 15 msec) with frequency-selective water suppression. The recorded signals in the time domain underwent the following postprocessing procedures: Gaussian filtering with maximum at 0 msec and half maximum after 150 msec, Fourier transformation, and constant and linear phase correction.

IMCL signals were measured as the area under the curve in fixed frequency borders (1.3–1.5 ppm) and compared to the methyl creatine signal (3.05–3.25 ppm). The creatine signal served as internal reference (maximum signal set to 3.15 ppm). IMCL values were calculated as the ratio of IMCL and creatine signal integrals.

Statistics

Dynamic IMCL changes (ΔIMCL) in the examinations (every 60 min, for a time period of 5 hr) are presented as percentage changes compared to the baseline value (IMCL value measured prior to the intervention) and are given as average ± SD in Fig. 1, and additionally as range in the text. The IMCL values of the study groups at different time points, as well as differences in ΔIMCL between the groups, were compared by a paired Student's *t*-test after passing a normality test. A P value < 0.05 was considered as statistically significant.

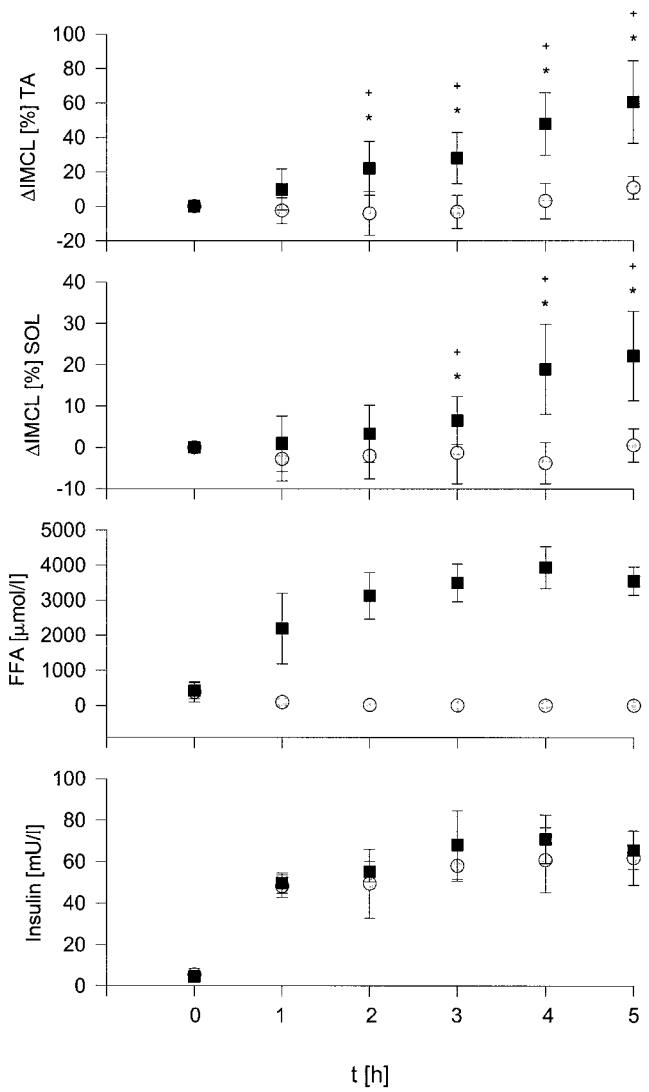

FIG. 1. Time course of ΔIMCL for TA and SOL, FFAs, and plasma insulin levels during hyperinsulinemia (circles) and hyperinsulinemia + lipid infusion (boxes). ΔIMCL is given as percentage change using the baseline value at t = 0 as reference. FFA concentration is given in µmol/l, plasma insulin levels in mU/l. SD is indicated as error bars for each time point. (+) indicates statistically significant differences in IMCL values within the group compared to the baseline with $P < 0.05$. (*) indicates statistically significant differences in ΔIMCL between the groups with $P < 0.05$.

RESULTS

IMCL Baseline Values

Baseline values were evaluated prior to the intervention at t = 0. IMCL values (given as the ratio of IMCL integral and

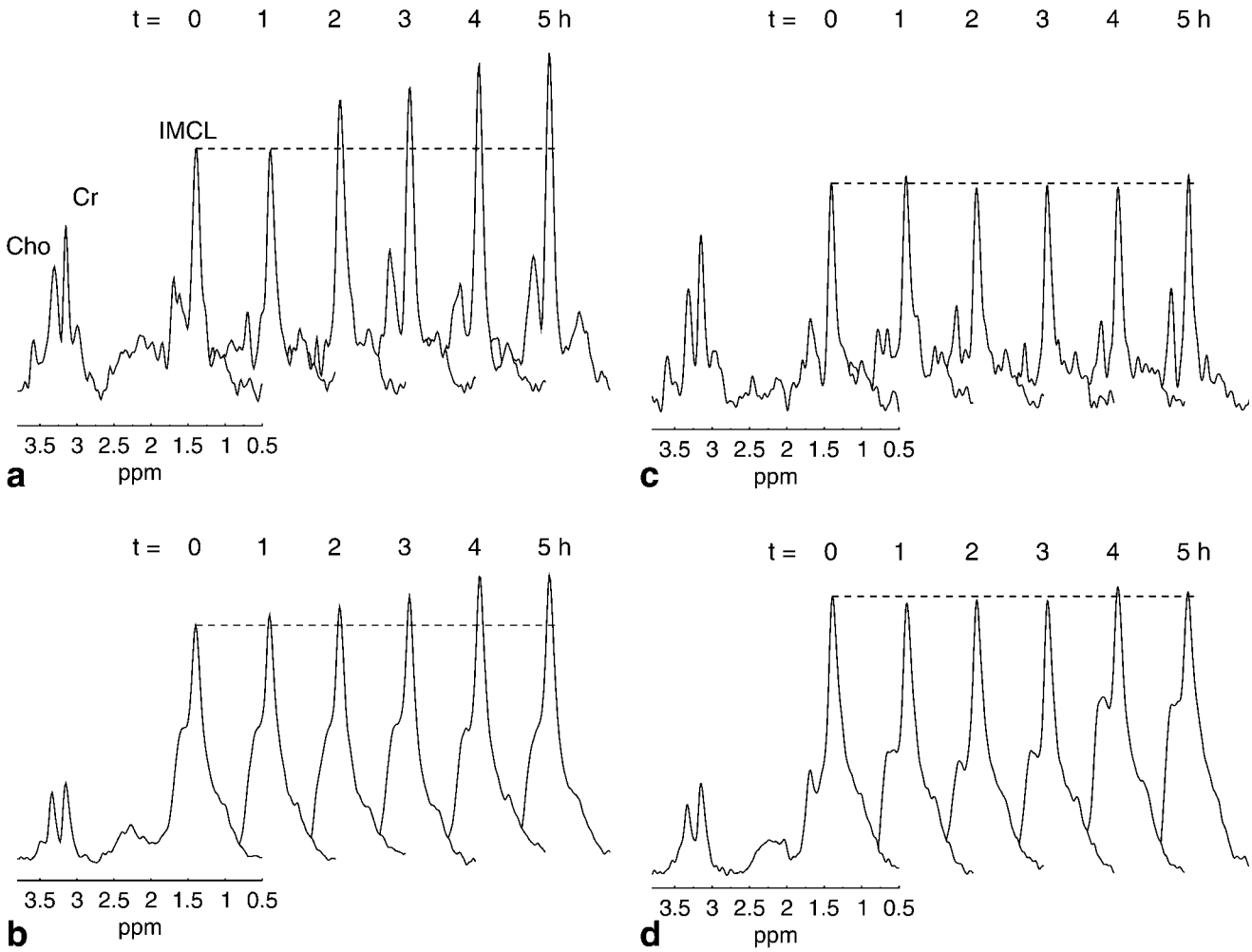

FIG. 2. Examples of ^{1}H-spectra of one subject at different time points for TA and SOL during hyperinsulinemia + lipid infusion (**a** and **b**), and during hyperinsulinemia alone (**c** and **d**). Signals are indicated as Cho (choline; 3.4 ppm), Cr (creatine; 3.15 ppm) and IMCL (1.3–1.5 ppm). Due to a better visualization, signals from 0.5–1.8 ppm (including the IMCL signal) are shifted to the right for the consecutive time points.

creatine integral) for the SOL were three- to fivefold higher compared to IMCL values in the TA for both groups (lipid infusion group: TA 2.6 ± 0.8 vs. SOL: 9.7 ± 2.4; control group: TA 2.2 ± 1.0 vs. SOL 10.1 ± 2.4). There were no significant differences in the baseline values between the groups ($P > 0.05$).

Time Courses of IMCL, Plasma FFAs, and Insulin

Time courses for IMCL changes of both muscles are presented in Fig. 1. Additionally, plasma values of FFAs and insulin are indicated for the different time points. During lipid infusion plasma, FFAs increased from a physiological level of 420 μmol/l (422.2 ± 228.0 μmol/l) to more than 3000 μmol/l (3125.8 ± 662.2 μmol/l) after the second hour. At the same time, plasma insulin rose from 5 mU/l (4.6 ± 2.9 mU/l) to a level of around 60 mU/l (55.3 ± 5.0 mU/l), and stayed at this value for the rest of the examination (Fig. 1).

In the control group, plasma insulin levels showed a similar time course as before. In contrast, FFAs were suppressed due to the high plasma insulin inducing a blockage of lipolysis.

During lipid infusion the IMCL content of the TA showed an elevation of more than 60% compared to the baseline (ΔIMCL 60.5 ± 24.0% vs. baseline, range: 31.3–84.9%; $P < 0.05$) after 5 hr. The time course of the IMCL values showed a slight increase of about 20% during the first 3 hr (first hour: 9.8 ± 11.9%, range: –10.8–18.2%, $P > 0.05$; second hour: 22.1 ± 15.6%, range: –0.1–41.5%, $P < 0.05$; third hour: 28.0 ± 14.8%, range: 5.0–45.1%, $P < 0.05$). After the fourth hour IMCL revealed an increase of almost 50% (47.7 ± 18.2%, range: 29.9–75.8%, $P < 0.05$) further rising to more than 60% above baseline after 5 hr, as reported. Figure 2a shows proton spectra of one subject, indicating an increase of IMCL for the TA.

In the control group, there were no significant changes of the IMCL values of the TA (total range of all values: TA: –15.1–17.8%; Fig. 2c). Compared to the control group, differences in ΔIMCL were significant at the time points t = 2, 3, 4, and 5 hr ($P < 0.05$; Fig. 1).

IMCL of the SOL revealed a similar time course, but the percentage changes were in a lower range and were not statistically significant up to the third hour (first hour: 0.9 ± 6.7%, range: –5.4–11.5%, $P > 0.05$; second hour: 3.3 ± 6.9%, range: –8.3–9.5%, $P > 0.05$; third hour: 6.5 ± 5.8%, range: –2.2–14%, $P < 0.05$). Similar to the TA, a more pronounced increase occurred after 4 hr with a ΔIMCL of 19% (18.9 ± 10.9%; range: 4.4–35.0%, $P < 0.05$), and after 5 hr with 22% (22.1 ± 10.8%; range: 4.1–32.9%, $P < 0.05$). As shown for the TA, IMCL values of the SOL did not reveal significant changes for the control group (total range of all values: SOL: –11.2–10.3%). Statistical significance in the difference of ΔIMCL between both groups was achieved after the third hour ($P < 0.05$; Fig. 1). Figure 2b and d shows proton spectra of the SOL, indicating an increase in IMCL with lipid infusion, whereas IMCL of the control group stayed around the baseline value at t = 0.

DISCUSSION

The combination of an increased amount of circulating FFAs and a hyperinsulinemic state induced a significant increase of IMCL in both muscles. While IMCL in the mainly oxidative SOL was only 20% higher after 5 hr, IMCL in the mixed TA had a more marked relative increase of 60%. On the other hand, the absolute increase of the IMCL content during the time course is expected to be even higher in SOL, since SOL had a clearly higher baseline IMCL level (about three- to fivefold compared to TA, as reported in this study and Ref. 17).

The results suggest a specific time pattern in the elevation of IMCL. In both muscles (TA and SOL) ^{1}H-MRS revealed minor changes during the first 3 hr of the experiment, whereas between the third and fourth hour the most pronounced increase in IMCL occurred. As shown in the control group, hyperinsulinemia per se did not have any significant influence on IMCL and lead to a suppression of plasma FFA levels. Furthermore, Krssak et al. (24) recently reported that elevation of circulating FFAs without hyperinsulinemia did not alter IMCL levels. Our findings are in accordance with the observations of Chalkley et al. (23), who found an increase of the triglyceride content in the red gastrocnemius muscle of the rat under similar conditions, i.e., after a 5-hr hyperinsulinemic glucose clamp with the infusion of a lipid emulsion and heparin. Dynamic changes of the fat content have not been reported from the rat study because the whole muscle was required for the analysis.

In contrast, volume-localized ^{1}H-MRS not only allows the measurement of muscular lipids at different time points, it can also differentiate between IMCL and EMCL, which cannot be obtained by a muscle biopsy. There is evidence that IMCL is the metabolically relevant lipid compartment of muscle lipids (21,22).

To our knowledge, this is the first human study that shows a rapid elevation of IMCL within a short (5-hr) time period. The results indicate that a short-term increase in IMCL can be induced by the combination of hyperinsulinemia and elevation of circulating FFAs, but not by one of these conditions alone.

By using ^{1}H-MRS as a noninvasive tool, alterations of IMCL can be easily observed under various conditions. The assessment of IMCL and its regulation allows new insight into the metabolic activity of this lipid compartment, especially in the context of insulin resistance and type 2 diabetes (16–19,21,22).

In conclusion, this study shows for the first time that a short-term elevation of circulating FFAs and hyperinsulinemia has a prompt effect on the formation of IMCL in humans. These changes can be monitored elegantly and noninvasively by ^{1}H-MRS.

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