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SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 522–528

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Age and muscle-type modulated role of intramyocellular lipids in the progression of insulin resistance in nondiabetic Zucker rats

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Received 21 November 2004; revised 29 November 2004; accepted 29 November 2004

Abstract

The effect of muscle fiber type and maturation on intramyocellular lipid (IMCL) content and its relationship to insulin resistance was investigated. Intramyocellular lipid content in slow-twitch (soleus) and fast-twitch (tibialis anterior, TA) muscles of *fa*/*fa* (Zucker fatty rat, ZFR) and age-matched lean (Zucker lean rat, ZLR) Zucker rats were repeatedly measured over 3 months. Intramyocellular lipid levels in both the soleus and the TA were significantly higher in the ZFR relative to the ZLR. For the ZFR, $IMCL_{TA}$ increased by ~2-fold from 5.3 to 8.4 weeks of age. No subsequent accumulation of $IMCL_{TA}$ occurred in ZFR from 8.4 up to 13.1 weeks of age. For ZLR, $IMCL_{TA}$ contents steadily decreased from 6.6 to 13.1 weeks of age (−77%, $P < .05$). In contrast, IMCL levels in the soleus were not significantly altered in either rat strain over the course of the study. Maximum impairment in whole-body insulin sensitivity in ZFR was observed at 9-weeks of age, concomitant with peak $IMCL_{TA}$ accumulation. Insulin-stimulated 2-deoxy-D-glucose (2DG) transport in the TA muscle of 10.2- and 14.1-week-old ZFR was significantly impaired relative to age-matched ZLR. Insulin-stimulated glucose uptake in the soleus of ZFR and ZLR decreased ($P < .05$) as the animals matured (ZFR, −49%; ZLR, −69%). Overall, these results support the hypothesis that fast-twitch glycolytic muscles play a major role during the onset of insulin resistance. In addition, proper timing may govern the success of a pharmacological studies aimed at measuring the impact of insulin-sensitizing drugs on IMCL.

1. Introduction

The intracellular accumulation of muscle lipid as droplets in diabetic animals has been known for some time now [1,2]. More recently, elevated levels of intramyocellular lipids (IMCLs) have been associated with an inability to sustain peripheral glucose disposal [3-5]. The mechanism by which myocellular lipids may exert such deleterious effects on muscle insulin sensitivity remains unknown.

A number of in vivo studies have shown that IMCL content, as opposed to the extramyocellular lipids (EMCLs), can be used as a marker of insulin resistance (IR) [6-8]. In human beings, there is a strong negative correlation between IMCL content and insulin sensitivity, as estimated from the *M* value indicative of the insulin-stimulated whole-body glucose uptake [6-9]. Recent data also support the fact that IMCL accumulation may reduce insulin-stimulated muscle glucose uptake through an inhibition of glucose transport activity [10], and hence may be a primary cause of IR. However, studies using diabetic Zucker rats have shown that IMCL contents decrease with age in spite of an increase in IR [11,12]. Even more intriguing is the fact that aerobic/anaerobic training seems to stimulate storage of fasting IMCL in thigh muscles while improving insulin sensitivity [13].

Nondiabetic *fa*/*fa* Zucker rats may exhibit signs of glucose intolerance by the age of 7 to 8 weeks. Maximum glucose intolerance in these rats is usually reached by 12 to 13 weeks of age [14]. In Zucker diabetic rats, peripheral IR is also clearly present at the age of 7 weeks and their circulating glucose levels usually start exceeding normal limits shortly thereafter [15]. In fact, in 7- to 8-week-old Zucker diabetic rats, a seemingly inevitable transition to overt diabetes seems to occur in this late stage model of type II diabetes [15]. This is due, at least in part, to decrease in insulin production, concomitant with a loss of β-cell mass [16]. However, the continuous decline in plasma insulin

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0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.11.006

concentration [15] may not be sufficient on its own to explain the reduction in glucose uptake. Fasting levels of plasma insulin being also above normal in nondiabetic 5- to 6-week-old *fa*/*fa* Zucker rats [17], other factors could play a significant role in the progressive development of peripheral IR. This has led to the suggestion that IMCL accumulation may also act as a possible negative effector on muscle glucose use.

On the assumption that the occurrence of IR depends on both muscle fiber type and maturation, the present study explored time-course changes in IMCL of slow-twitch and fast-twitch muscles in ZLR and ZFR. In particular, our intention was to evaluate the relative contribution of IMCL stores in oxidative and glycolytic muscles to the development of peripheral IR. To this end, age-related impairment of whole-body and muscle insulin sensitivity were assessed in parallel using a separate cohort of ZLR and ZFR. These latter measurements were performed at critical time points as determined from the IMCL study. Once documented, such data may prove useful especially when designing pharmacological studies on antidiabetic drugs and using IMCL as an in vivo biomarker of peripheral IR.

2. Methods

2.1. Animals

The study design is summarized in Fig. 1. Intramyocellular lipid measurements were conducted on a cohort of male *fa*/*fa* Zucker (ZFR, 145 ± 5 g at 5.3 weeks of age, n = 6) and lean Zucker (ZLR, 145 ± 3 g at 6 weeks of age, n = 6) rats. Muscle lipid levels were measured when the rats were 5.3 (ZFR only), 6.6, 8.4, 10.3, 11.7, and 13.1 weeks of age. During nuclear magnetic resonance (NMR) data acquisition, animals were anesthetized for approximately 45 minutes

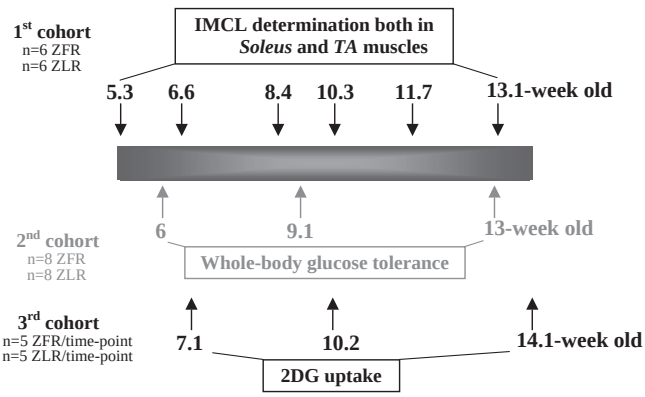

Fig. 1. Protocol timelines. Muscle lipid contents were repeatedly measured in a first cohort of rats, both in the TA and the soleus muscle of each animal, every 1 to 2 weeks from 5.3 to 13.1 weeks of age. Except for the 5.3-week time point missing in the ZLR subgroup, IMCL content were measured in age-matched ZLR and ZFR. Whole-body glucose tolerance was measured in a second cohort of rats of 6, 9, and 13-week of age using the OGTT. Each animal served as its own control. Muscle insulin-stimulated glucose uptake was determined at similar time points from a third cohort of rats (n = 5/time point/subcohort) using the 2DG method.

Table 1
Major fatty acid components of the 12% fat energy chow diet

Major fatty acids	As % of the diet (4.5% total fat)	As % of total fat
C16:0	0.7	16
C18:0	0.2	4
C18:1	0.9	20
C18:2	2.0	44
C18:3	0.2	4

with 2% isoflurane, and their body temperatures were kept at 37°C ± 1°C using warm air. Time-course changes in whole-body IR (glucose tolerance) were measured in a second cohort of ZLR (n = 8) and ZFR (n = 8) at the age of 6, 9.1, and 13 weeks using the oral glucose tolerance test (OGTT). Using a third cohort of animals, insulin-stimulated glucose uptake by the tibialis anterior (TA) and soleus was measured at specific time points, that is, 7.1-, 10.2-, and 14.1-week-old animals (n = 5 ZLR and n = 5 ZFR/time point) via the 2-deoxy-D-glucose (2DG) transport method [18]. The time points selected were determined a priori, on the basis of IMCL data obtained in the first cohort of rats. All animals had free access to a normal chow diet (12% fat energy from Harlan Teklad Rodent, diet 8604) throughout the 8-week study. This diet is predominantly plant based, but does contain 2 animal products, fish meal and dried whey. The overall fat level of the diet is about 4.5%. Of this, about one third comes from the added soybean oil. The major fatty acids present in the diet are given in Table 1. Experimental procedures were carried out in compliance with the guidelines of the Novartis Institutional Animal Care and Use Committee.

2.2. Oral glucose tolerance test on rats and analytical procedures

After a 12-hour fast, rats were administered a 1.0 g/kg glucose bolus orally, and blood samples were obtained via a tail nick at 0, 15, 30, 60, and 120 minutes after glucose administration. Blood samples (200 μL) were collected in heparinized microcentrifugation tubes (Brinkmann Instruments, Inc, Westbury, NY). Samples were immediately centrifuged (10 000 rpm at 4°C for 5 min) and measured for plasma glucose concentrations using a YSI 2700 Dual Channel Biochemistry Analyzer (Yellow Springs Instrument Co, Yellow Springs, Ohio). Plasma insulin concentrations were measured using an ELISA assay kit (American Laboratory Products Co, Windham, NH).

2.3. Determination of insulin-stimulated muscle glucose transport

2DG transport was measured in TA (fast-twitch) and soleus (slow-twitch) muscles to identify in vivo age-related impairment in insulin-stimulated glucose uptake. Once transported into the cell and phosphorylated by hexokinase, 2DG is not further metabolized, hence making it a good marker for glucose transport [18]. In this experiment,

mannitol was also used in the preparation to correct for the extracellular space while calculating 2DG intracellular concentration. Mannitol has a similar molecular weight as 2DG but cannot enter the cell because the lack of a transport system to go across the cell membrane.

Within 1 week before the actual measurement, all rats were cannulated in the jugular vein. To carry out measurements under 4-hour fasting conditions, food was removed at 6:00 AM on the day of the experiment. At 9:00 AM, rats were placed in plastic cages and their cannulas hooked to a saline line. At 9:45 AM, a basal blood sample (80 μL) was taken for plasma glucose and insulin measurements. Then, cannulas were flushed with 0.2 mL of heparinized saline (100 U/mL). At 10:00 AM (time, 0 minute), ^{3}H-2-deoxyglucose (60 μCi/kg), ^{14}C-mannitol (30 μCi/kg), and insulin (0.1 U/kg) were given as bolus injections. Blood samples (120 μL) were taken at 2, 5, 10, 15, 20, 30, and 45 minutes after the bolus injection. Plasma samples were stored at -20°C after glucose concentrations were measured. Immediately after the 45-minute blood sample collection, animals were euthanized with an overdose of sodium pentobarbital through the cannula. Muscle tissues (soleus and TA) from the right leg of the animals were dissected out within 5 minutes posteuthanasia, clamp frozen, and stored at -80°C until being processed.

The injection solution (1 mL/kg) was freshly prepared for each experiment. For each 1 mL of injection solution, 0.06 mL of ^{3}H-2-deoxyglucose stock solution (1 mCi/mL) (dried out overnight) and 0.3 mL of ^{14}C-mannitol stock solution (0.1 mCi/mL) were added to 0.63 mL of saline with 0.1% bovine serum albumin. Then, 10 μL of 1:10 diluted insulin (10 U/mL after dilution, 10 μL containing 0.1 U insulin) was added to this solution. The final ^{3}H concentration was 0.06 mCi/mL, the ^{14}C concentration was 0.03 mCi/mL, and the insulin concentration was 0.1 U/mL.

2.4. Analytical procedures

Plasma samples were prepared by the addition of 25 μL of 10% tricyclic antidepressant to 25 μL of plasma. The samples were then vortexed and centrifuged. Ten microliters of supernatant was pipetted into a scintillation vial in duplicates. Five milliliters of scintillation fluid was then added and this new solution was shaken before counting. Muscle tissues were homogenized in 10% tricyclic antidepressant (volume [mL] = tissue wt [g] × 10). After centrifugation, 100 μL of the supernatant was added to 5 mL of scintillation fluid in duplicates for final counting. Finally, 5 μL of injection solution was added to 5 mL of scintillation fluid in quadruplets. Each vial was counted for 1 minute in a Beckman Scintillation counter (Model LS3801; Beckman Instruments, Irvine, Calif) using a dual-labeled counting program. 2DG transport activity was calculated according to the following equation:

$$2DGT(\text{nmol/g/min}) = [Gp \times Ct_{45}] / \left[\int_0^{45} Cp(t)d(t) \right]$$

where Gp is the average plasma glucose concentration (mmol/L) measured over 45 minutes after tracer injection, Ct_{45} corresponds to the intracellular ^{3}H counts (disintegrations per minute [dpm]/g) in tissue collected at 45 minutes after tracer injection, and $\int_0^{45} Cp(t)d(t)$ is the area under the plasma ^{3}H curve (dpm/mL) over the 45-minute postinjection period. Of note, Ct_{45} includes a correction for tissue extracellular space on the basis of the ratio of ^{14}C to ^{3}H counts in the tracer solution and ^{14}C counts in tissue samples collected at 45 minutes.

2.5. In vivo localized ^{1}H-magnetic resonance spectroscopy

All in vivo magnetic resonance measurements were performed on a Bruker Avance 3.0 T/60 cm wide-bore instrument (Bruker Medical, Billerica, MA) equipped with a 12-cm id actively shielded gradient insert and a B-S20 shim system. Intramyocellular lipid data were obtained using both a 72-mm birdcage resonator (transmitter) and a 2.0-cm surface coil (receiver) in a cross-coil fashion. Anesthetized rats were positioned prone with the left leg placed on top

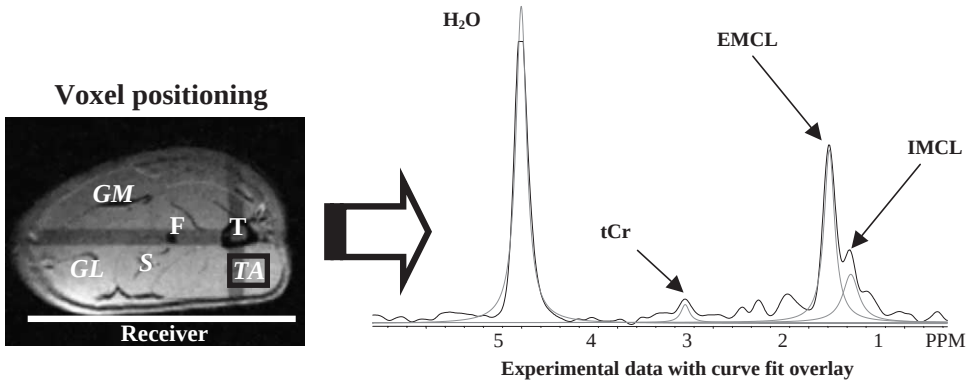

Fig. 2. Localized ^{1}H-NMR spectroscopy. Position of the 8-mm^{3} voxel was carefully adjusted either on the TA or the soleus muscle from a previous series of scout images in all orientations (transverse section shown here). Spectral analysis then was performed in such a way that resonances of tCr (3.02 ppm), EMCL (1.5 ppm), and IMCL lipids were fitted as singlets. GM indicates gastrocnemius medialis; GL, gastrocnemius lateralis; F, fibula; T, tibia; S, soleus.

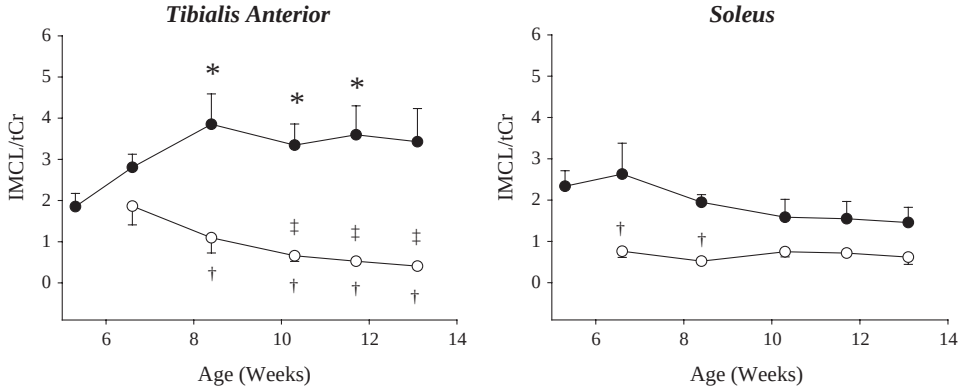

Fig. 3. Time-course changes in the IMCL/tCr ratio as measured in the left TA and soleus muscles of ZLR and ZFR. One-way repeated measures ANOVA showed that the IMCL/tCr increase measured in ZFR_{TA} over the first ~3 weeks of the investigation period and the continuous decrease measured in ZLR_{TA} were both statistically significant ($P < .05$). Asterisk indicates $P < .05$ vs ZFR baseline (5.3 weeks); double dagger, $P < .05$ vs ZLR baseline (6.6 weeks); dagger, $P < .05$ vs ZFR.

of the surface coil within the isocenter of the magnet. Transverse, sagittal, and coronal scout images were acquired to carefully position the 8-mm^3 volume of interest in the left TA and soleus muscles, avoiding blood vessels and gross adipose tissue deposits (Fig. 2). Localized ^{1}H-magnetic resonance spectra were obtained using a PRESS sequence (echo time of 18 milliseconds, repetition time of 2 seconds, 4096 data points over a 6-kHz spectral width, CHESS water suppression, 1000 scans, voxel of 2 × 2 × 2 mm^3). Before the acquisition, the magnetic field was shimmed on localized water signal using Fastmap [19] to achieve typical line widths of approximately 11 Hz. Spectra were processed using the Nuts-PPC software package (AcornNMR, Inc, Fremont, Calif). Once spectra were line broadened, phased, and baseline corrected, peak areas for total creatine (tCr, 3.02 ppm), EMCL (methylene peak at 1.5 ppm), and IMCL (methylene peak at 1.3 ppm) were determined using a line-fitting procedure (see Fig. 2). Intramyocellular lipid content was then expressed as a percentage of tCr content. Good sensitivity of the NMR signal was assessed from a systematic measurement of signal-to-noise ratio SNR as determined both from the tCr peak and lipid peaks and using the following equation:

$$\mathrm{SNR} = [2.5 \times (\text{peak height})]/\text{peak} - \text{to} - \text{peak noise}$$

2.6. Statistical analysis

All values are expressed as means ± SEM. Intergroup comparisons and time-course variations were analyzed using Student *t* tests and analysis of variance (ANOVA) followed by a Tukey post hoc test when appropriate. A $P < .05$ was considered statistically significant.

3. Results

At 6 weeks of age, ZFR and ZLR had similar body weights. At this age, plasma glucose levels were similar between the 2 rat strains (ZFR 102.5 ± 7.2 vs ZLR 99.7 ± 3.7 mg/dL, NS). However, 6-week-old ZFR displayed signs of IR as indicated by a mild hyperinsulinemia relative to the ZLR rats (ZFR 47.1 ± 4.6 vs ZLR 10.5 ± 1.0 mg/dL,

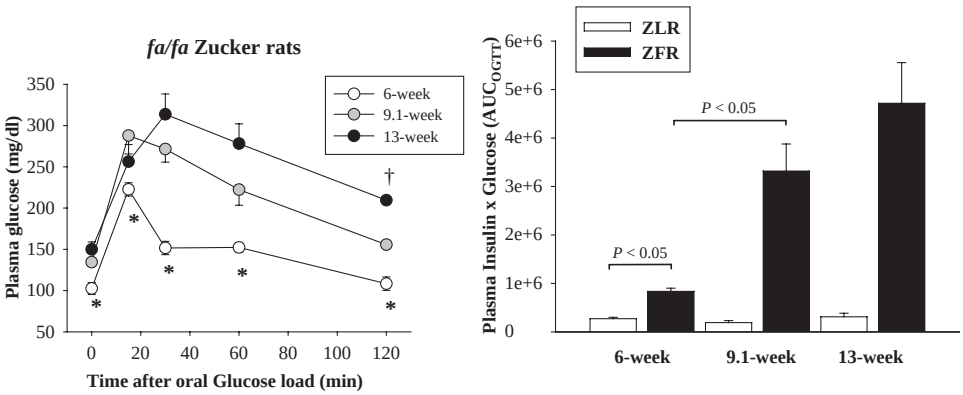

Fig. 4. Glucose excursion during OGTT measured in ZFR over a 7-week period (raw data in the left panel). Area under the curve values for the glucose × insulin product calculated in ZFR and ZLR from 6 to 13 weeks of age (right panel). Asterisk indicates $P < .05$, 6 weeks vs 9.1 and 13 weeks; dagger, $P < .05$, 9.1 vs 13 weeks.

$P < .05$). Intramyocellular lipid levels measured in the soleus muscle ($IMCL_{soleus}$) of 6.6-week-old ZFR were 3-fold greater relative to age-matched ZLR (Fig. 3, ZFR [IMCL/tCr] 2.63 ± 0.75 vs ZLR 0.76 ± 0.15, $P < .05$). However, IMCL levels measured in the TA muscle ($IMCL_{TA}$) were not significantly different between the 2 strains (ZFR [IMCL/tCr] 2.81 ± 0.31 vs ZLR 1.86 ± 0.45, NS). Interestingly, the IMCL/tCr ratio was similar in both the soleus and the TA muscles of 5.3-week-old ZFR.

A marked accumulation of $IMCL_{TA}$, that is, approximately a 2-fold increase (Fig. 3), occurred by 8.4 weeks of age (1-way repeated measures ANOVA, time-effect $P < .05$) in the TA muscle of ZFR. No further accumulation of $IMCL_{TA}$ occurred in ZFR up to 13.1 weeks of age. In contrast, $IMCL_{TA}$ content in ZLR steadily decreased throughout the 7-week measurement period (1-way repeated measures ANOVA, time-effect $P < .05$). No significant alteration of $IMCL_{soleus}$ occurred in either the ZLR or ZFR over the 7 weeks that IMCL was measured (ie, up to 13.1 weeks of age) (Fig. 3).

From 6 to 13 weeks of age, *fa*/*fa* Zucker rats remained normoglycemic under fasting conditions. However, a marked impairment of glucose tolerance (Fig. 4) was measured in ZFR subsequent to 9 weeks of age ZFR (ie, ~400% increase in plasma Insulin × glucose area under the curve, $P < .05$ vs 6 weeks). No further statistically significant increase in the plasma glucose excursion levels was observed in 13-week-old ZFR. By comparison, glucose tolerance in ZLR remained high and unmodified over the course of the 7-week investigation period. Given the inherent limitations of the OGTT, we also measured insulin-stimulated glucose transport in isolated TA and soleus muscles by the 2DG method (Fig. 5). In 7.1-week-old ZLR and ZFR, the rate of glucose transport was approximately 5-fold greater in soleus than in TA muscles ($P < .05$ for both strains). For ZLR, this gap in insulin-stimulated glucose uptake between the soleus and the TA muscle was no longer present as the animals matured (ie, in 10.2- and 14.1-week-old ZLR). For ZFR, the insulin-stimulated uptake of 2DG in the TA muscle decreased by approximately 50% as the animals aged from 7.1 to 14.1 weeks. Tibialis anterior muscle 2DG uptake in 14.1-week-old ZFR was 4-fold less ($P < .05$) than that measured in age-matched ZLR (Fig. 6). In contrast, for ZLR insulin-stimulated glucose transport in the TA muscle increased by approximately 110% as they matured from the age of 7.1 to 14.1 weeks ($P < .05$). A decrease in soleus 2DG uptake ($P < .05$ for time effect as tested with 2-way ANOVA) was measured in both the ZFR and ZLR as they aged from 7.1 to 10.2 weeks (ZFR, −49%, $P = 0.057$; ZLR, −69%, $P < .05$). No subsequent decrease in 2DG uptake was observed for either strain between 10.2 and 14.1 weeks of age. Interestingly, insulin-stimulated glucose uptake of the soleus muscle was greater for 10.2- and 14.1-week-old ZFR than for age-matched ZLR.

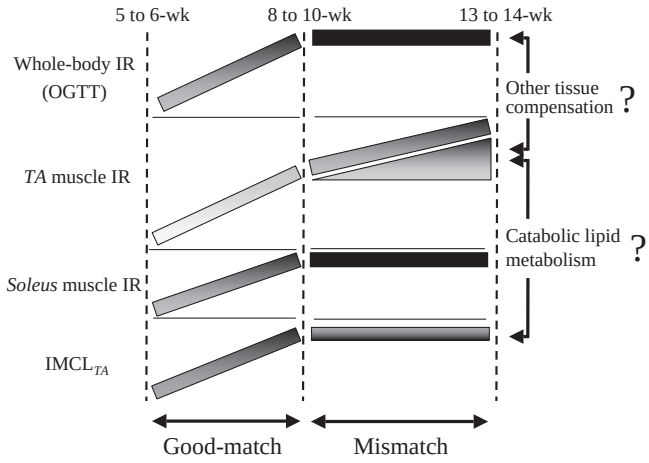

Fig. 6. Representation of age-associated relationships among whole-body glucose intolerance, muscle IR, and IMCL accumulation in *fa*/*fa* Zucker rats (ZFR). From 5 to 10 weeks of age, the development of glucose intolerance is concomitant with an increase in both muscle IR and IMCL in predominantly glycolytic muscles such as the TA. Beyond 10 weeks of age, IMCL content in fast-twitch muscles reaches a maximum level whereas insulin sensitivity continues to get further impaired (ie, mismatch). Because glucose intolerance remains constant during this time, other processes or tissues such as the liver may compensate for the insensitivity of peripheral glycolytic muscle to insulin stimulation.

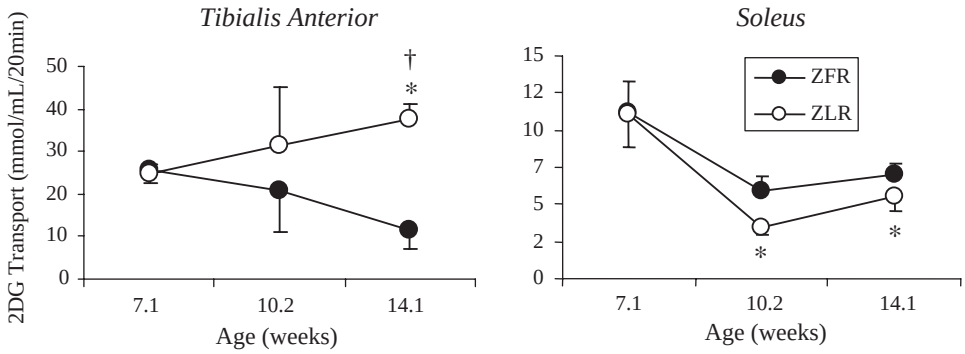

Fig. 5. Effect of age on insulin-stimulated muscle glucose transport, as assessed postmortem from the 2DG signal in tissue extracts. Measurements were performed in the TA (ie, glycolytic, fast twitch) and the soleus (ie, oxidative, slow twitch) muscles of ZLR and ZFR. Note that the rate of glucose uptake almost doubled in the TA muscle of 7- to 14-week-old ZLR compared to age-matched ZFR ($P < .05$ for time effect tested with 2-way ANOVA). Asterisk indicates $P < .05$, vs 7.1 weeks; dagger, $P < .05$, ZLR vs ZFR.

4. Discussion

In the present study, whole-body glucose intolerance as well as muscle specific IMCL content and insulin sensitivity were assessed in young ZFR and ZLR to gain insight into the role of muscle fiber type and maturation in the progression of glucose intolerance. In ZFR, whole-body glucose intolerance maximally develops by 9 weeks of age. Because skeletal muscle is responsible for approximately 80% of glucose disposal, the high glucose excursion values obtained most likely reflected an impairment of insulin-stimulated muscle glucose transport. Consistent with this, we observed an impairment of TA muscle 2DG-glucose uptake in ~10-week-old ZFR. $IMCL_{TA}$ increased 2-fold in ZFR as they matured from 5.5 to 8.4 weeks of age. No further accumulation of $IMCL_{TA}$ was observed beyond this age. Despite the absence of further IMCL accumulation, impairment of TA muscle insulin-stimulated glucose transport increased in 14-week-old ZFR. Interestingly, in the ZLR, an increase in insulin-stimulated glucose uptake in the TA muscle was concomitant with a decrease in IMCL storage during the 7-week period investigated. These results taken together provide additional support for the association of IMCL accumulation and insulin insensitivity in fast-twitch (glycolytic) muscles. In the present study, no association was found between IMCL and IR in slow-twitch (oxidative) muscles. Although $IMCL_{soleus}$ was 2-fold greater in 6-week-old ZFR compared to age-matched ZLR and $IMCL_{soleus}$ content for both strains remained stable over the course of the study, insulin-stimulated glucose uptake decreased over time to a similar extent for both rat strains.

The absence of further accumulation of $IMCL_{TA}$ simultaneous with an increased impairment of insulin-stimulated glucose uptake in the TA muscle of ~9- to 13-week-old ZFR agrees with recent data published by Kuhlmann et al [11]. In their study, Kuhlmann et al showed that IMCL accumulation in the TA muscle of Zucker diabetic fatty rats also reached a maximum at the age of 10 weeks. $IMCL_{TA}$ was maintained at a plateau level for the next 3 to 4 weeks, until the rats reached ~14 weeks. At subsequent time points, the $IMCL_{TA}$ levels decreased and the animals became overtly diabetic at the age of ~18 weeks, exhibiting fasting hyperglycemia and hypoinsulinemia.

The mismatch between muscle glucose uptake and $IMCL_{TA}$ accumulation in ~10- to ~14-week-old ZFR may indicate that lipid-induced muscle IR no longer holds in more mature rats. A decrease in muscle lipoprotein lipase (LPL) activity that would reduce the uptake and storage of circulating fatty acids in fast-twitch skeletal muscles of ZFR may however explain the absence of further $IMCL_{TA}$ accumulation [20,21]. A relationship between impaired muscle fatty acid oxidation and IR has been found in normal animals as they get older [22]. It is therefore possible that a similar impairment of muscle fatty acid oxidation occurred within the *fa*/*fa* Zucker rat with the result that between ~9 and 13 weeks of age $IMCL_{TA}$ remained constant. The reason for the decrease in $IMCL_{TA}$ in ZLR animals is unclear. In non–insulin-resistant rats, LPL activity has been reported to decrease in fast-twitch muscles as the animals mature [23]. This would limit $IMCL_{TA}$ accumulation in the ZLR, and combined with normal fatty acid oxidation, this may be responsible for the decrease in $IMCL_{TA}$.

The difference in the soleus and TA insulin sensitivity likely is the result of their specific metabolic profiles. Slow-twitch muscles mostly rely on cellular lipid storage as a preferential substrate for contraction, unlike fast-twitch muscles that rely mostly on carbohydrates. Slow-twitch and fast-twitch muscle fibers also exhibit fundamental differences in their capacity to synthesize lipids. For instance, the triacylglycerol synthesis rate increases in proportion to the oxidative capacity of skeletal muscle fibers, but independently of blood flow conditions [24]. In addition, both insulin responsiveness and capacity to oxidize fatty acids are greater in slow-twitch muscles compared to fast-twitch muscles [25,26]. Therefore, not surprisingly, metabolic disorders such as type II diabetes have also been linked to an increased amount of glycolytic fibers at the expense of oxidative fibers [27]. Finally, although not reported elsewhere, a defect in the inhibitory action of insulin on lipid synthesis in predominantly glycolytic muscles of insulin resistant animals is also possible. Along these lines, the nature of triglyceride-derived metabolites that are thought to interfere directly with insulin action (eg, long-chain fatty acyl-CoAs) [28,29] may actually vary between oxidative and glycolytic muscles and thereby could contribute differently to the development of IR.

One of the goals of the present work was to delineate the limitations of using IMCL as a biomarker of muscle IR in a pharmacologically relevant model. In addition to animal age, the present work highlights the necessity of selecting predominantly glycolytic muscles to observe a measurable accumulation of IMCL without resorting to high-fat diets. As shown in Fig. 6, in the *fa*/*fa* Zucker rat model, a good association between IMCL accumulation and peripheral IR is found (i) when animals are relatively young (<12 weeks old) and (ii) in a predominantly fast-twitch muscle such as the TA muscle. In pharmacological studies, initial baseline measurements should be made before the animals are older than 6 weeks of age. Finally, IMCL measurement may be relevant for only certain models of IR. The relationship between IMCL accumulation and glucose intolerance or impairment of glucose uptake may no longer hold in transgenic models of diabetes or IR where the insulin receptor is antagonized or the insulin signaling pathway is disrupted.

Our data may not be applicable for clinical studies. In human beings, IMCL content in the soleus has been reported to be 2-fold greater than in the TA muscle [30]. In addition, IMCL levels are very sensitive to genetic background, exercise, and diet, which can vary greatly in human subjects [31,32]. In the present study, we found that in young rats, IMCL/tCr levels in the soleus and the TA muscles are similar, consistent with previous literature [11,12]. Therefore,

our findings should be considered only in the context of pharmacological rodent models.

In conclusion, although the mechanism by which glucose intolerance develops still needs to be further elucidated, a defect in glycolytic/mixed muscle lipid metabolism may play a prevalent role. The accumulation of IMCL in glycolytic/mixed muscle can be used as a biomarker of peripheral IR in the *fa*/*fa* Zucker rat. However, animal age and muscle type should be considered when designing pharmacological studies involving rodents.

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