Proton magnetic resonance spectroscopy shows lower intramyocellular lipid accumulation in middle-aged subjects predisposed to familial longevity


Department of Gerontology and Geriatrics; C. J. Gorter Center for High Field MRI, Department of Radiology; Department of Internal Medicine; Netherlands Consortium for Healthy Ageing; and Department of Medical Statistics, Section of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Submitted 30 August 2011; accepted in final form 10 November 2011

METHODS

Study design and population. The LLS comprises 421 families, as described more extensively elsewhere (24). Families were included and regarded as predisposed to familial longevity if at least two long-lived siblings were alive and fulfilled the age criterion of 89 yr or older for males and 91 yr or older for females. Because no proper controls exist for this age group, for further studies the offspring of these long-lived nonagenarians were included with their partners as controls. The offspring carry on average 50% of the genetic propensity of their long-lived parent and were shown to have a 30% lower mortality rate compared with their birth cohort (24). Their partners, with whom most have had a relationship for decades, were included as environmentally matched controls, thereby minimizing environmental differences between the groups under study. We have shown previously that major lifestyle factors, such as smoking and physical activity, do not differ between groups (20). There were no selection criteria on health or demographic characteristics. In total, 1,671 offspring and 744 controls were included in the LLS. For the present study, participants were selected from a previous LLS (sub)cohort and invited by mail. Exclusion criteria were the presence of diabetes earlier research has shown an enhanced or preserved insulin sensitivity in centenarians (14) and in the offspring of nonagenarians in the Leiden Longevity Study (LLS) (33). The LLS has been designed to identify factors related to human longevity. The LLS consists of 421 long-lived families; these families are comprised of multiple nonagenarian siblings and their offspring, the partners of the offspring are included as controls. In the LLS, the middle-aged offspring show a lower prevalence of age-related diseases compared with controls (32). Moreover, the offspring from long-lived nonagenarian siblings show a lower prevalence of metabolic syndrome and better glucose tolerance, as well as a 25% increased peripheral insulin sensitivity, that could not be explained by differences in body composition or lifestyle (21, 33).

Several candidate mechanisms, other than differences in body composition, may explain the observed difference in insulin sensitivity between subjects predisposed to longevity and controls. Independent of generalized adiposity, lipid accumulation within muscle cells (intramyocellular lipids (IMCL)) is one potential candidate. A negative correlation between IMCL levels and insulin sensitivity has been found among young, lean, and healthy subjects (22). Moreover, higher IMCL levels were found in lean, insulin-resistant offspring of T2DM patients compared with lean controls (18). In this study, we used noninvasive proton magnetic resonance spectroscopy (MRS) to examine whether offspring of long-lived nonagenarian siblings and their partners from the LLS, who differ in insulin sensitivity, have different IMCL levels.

IN WESTERN SOCIETIES, the proportion of elderly people continues to increase, with a concomitant increase in the prevalence of various syndromes and diseases related to old age. Examples include metabolic syndrome and type 2 diabetes mellitus (T2DM), both of which are characterized by insulin resistance (4). Insulin resistance increases with age (5), contributing to the higher incidence of T2DM in old age. Furthermore, insulin resistance has been associated with various other age-related pathologies such as cardiovascular disease, cancer, and Alzheimer’s disease (3).

The association between insulin resistance and a variety of age-related diseases suggests that preservation of insulin sensitivity in old age might be beneficial for healthy aging. Indeed,
mellitus, use of glucose-lowering medication, or contraindications for 7 Tesla (7T) MRI and MR spectroscopy measurements. We selected our participants from a pool of 370 individuals who had recently participated in a 3T MR research within the LLS. Because we wanted to compare offspring with their partners, we selected only complete couples. This narrowed the subgroup of 370 down to 288 subjects. Criteria for 7T are more strict than for 3T and exclude virtually all subjects with the presence of ferromagnetic material. We aimed to include 30 couples; to achieve this, we invited 62 couples based on prior information on surgical history, selecting those with as little surgery as possible. Of these 62 couples, 15 were not interested in participating in the study, 13 were found not to be eligible for 7T MRI because of the presence of ferromagnetic implants after more specific investigation of the surgical history, and five were not eligible because of the presence of diabetes. Finally, 29 couples were included in the study. The Medical Ethics Committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all participants.

1H-MRS. Subjects were invited to the study center for the MRS measurement at 9 AM after an overnight fast. Subjects were instructed not to perform any moderate or strenuous exercise 2 days prior to the study day. MR spectra were measured in the left tibialis anterior muscle on a Philips Achieva MRI scanner at a field strength of 7T (Philips Healthcare, Best, The Netherlands). A custom-built quadrature half-volume transmit-and-receive coil was used for all measurements (31). Subjects were positioned supine and feet first in the MRI scanner. Transverse and sagittal T1-weighted images of the lower leg were acquired for voxel placement. The voxel was carefully placed to avoid vasculature, tendon plate, subcutaneous, and visible interstitial fat (Fig. 1). MR spectra were acquired using a stimulated echo acquisition mode sequence with an echo time of 22 ms, a mixing time of 17 ms, a voxel size of $10 \times 10 \times 10$ mm, and repetition time of 2,000 ms and variable pulse power and optimized relaxation delays water suppression. Shimming was performed using a B0 map (23), and the 90° pulse was determined using volume selective power optimization (31).

Data analysis. 1H-MR spectral analysis was performed with the Java-based Magnetic Resonance User Interface (jMrui) software package (29). MR spectra were fitted to a Lorentzian line shape using a nonlinear least-squares algorithm (AMARES; Fig. 2). Values were corrected for partial saturation using T2 relaxation times of 91.6 ms for creatine and 66 ms for IMCL (19), assuming negligible effects of residual dipolar coupling at this short echo time (8). IMCL levels were calculated by normalizing the signal intensity of the CH2 resonance of IMCL to the total creatine (tCr) signal, thereby assuming constant tCr levels between groups, a commonly used and verified method (12, 13, 30). The tCr signal intensity was determined by the sum of twice the upfield signal of the creatine CH3 triplet at 3.1 ppm plus the main CH3 signal at 3.02 ppm (peaks 5 and 6 in Fig. 2); this calculation of tCr was performed to avoid possible contamination of the downfield creatine CH3 peak by the extramyocellular lipid (EMCL) fat pool at 2.95 ppm (19).

Fasted glucose. Self-monitored blood glucose was performed by participants four times daily before meals and bedtime for 3 consecutive days using a standard blood glucose monitor (Bayer Contour). Fasted blood glucose was determined by the mean of the three prebreakfast self-monitored blood glucose measurements. Data on fasted blood glucose were available for 38 participants.

Hyperinsulinemic euglycemic clamp study. A hyperinsulinemic euglycemic clamp study was performed as described in more detail earlier (33). Data on clamp study parameters were available for 11 participants.

Anthropometric data. Height and weight were measured, and body mass index (BMI) was calculated by dividing weight (in kg) by the square of height (in m).

Physical activity. Physical activity was assessed by the short International Physical Activity Questionnaire (IPAQ) (7). A continuous activity score was expressed as metabolic equivalent levels—minutes per week.

Statistical analysis. Differences between offspring and controls were compared using an analysis of variance with correction for age, sex, BMI, and physical activity. A pairwise Student t-test was used to compare differences in IMCL/tCr ratios between couples. A P value of <0.05 was considered statistically significant. All analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL).

RESULTS

Of the 29 couples, five were excluded because one member of the couple did not complete the MRS measurement; two participants did not finish the MRS measurement due to claustrophobia, and for three participants measurements were excluded due to instrumental failures. Twenty-four couples (48 subjects) completed the study and were included in the final analyses.

Baseline characteristics are shown in Table 1. No significant differences were found in age, BMI, or IPAQ score, although offspring were slightly older, had a slightly lower BMI, and were somewhat less active compared with their partners. Sex distribution tended to differ between groups ($P = 0.08$). However, IMCL levels, expressed as IMCL/tCr ratio, were similar between males and females ($3.6 \pm 0.5$ vs. $4.0 \pm 0.5$, $P = 0.53$). To assess whether the contrast in glucose metabolism was present between groups, we compared available fasted glucose levels and, in a small subset, parameters derived from a hyperinsulinemic euglycemic clamp study. As ex-
expected, offspring had lower fasted blood glucose levels compared with their partners as well as a higher whole body insulin sensitivity (M value) and glucose disposal rate (GDR).

Figure 3 shows the IMCL/tCr ratio for both groups and paired couples of offspring and their partners. Because IMCL levels can be influenced by many environmental factors, we calculated differences in IMCL levels between groups, with adjustment for age, sex, BMI, and physical activity score. The mean IMCL/tCr ratio was borderline significantly lower in the group of offspring vs. the group of their partners (3.1/4.5 vs. 4.5/6.0, P = 0.051; Fig. 3A). In a paired analysis, which we performed to further exclude residual confounding factors, offspring had significantly lower IMCL levels compared with their partners (P = 0.038; Fig. 3B). Furthermore, IMCL data were positively associated with fasted glucose levels in a subset (n = 38) of the population, with a 1.68 (± 0.77, SE) mean increase in IMCL/tCr ratio for every 1 mmol/l increase in fasted glucose (P = 0.036 with adjustment for age, sex, BMI, and physical activity score).

**DISCUSSION**

In this study, we show that middle-aged individuals predisposed to longevity have lower IMCL levels in the tibialis anterior muscle compared with their partners. This study is the first to show such a difference among nondiabetic, middle-aged subjects.

Earlier research has shown that IMCL levels can be influenced by various factors such as weight, age, sex, exercise, and

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Offspring</strong></td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Females, n (%)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Ln IPAQ score, METs</td>
</tr>
<tr>
<td>Glucose metabolism</td>
</tr>
<tr>
<td>Fasted glucose, mmol/l*</td>
</tr>
<tr>
<td>M value, mg·kg⁻¹·min⁻¹†</td>
</tr>
<tr>
<td>Glucose disposal rate, μmol·kg⁻¹·min⁻¹†</td>
</tr>
</tbody>
</table>

Continuous data are represented as means ± SD; n = 24. IPAQ, International Physical Activity Questionnaire, expressed as metabolic equivalent score (METs). *Data available for 38 subjects. †Data available for 11 subjects. Fasted glucose represents mean value of 3 self-monitored fasted capillary blood glucose samples on 3 consecutive days. Data on glucose metabolism were adjusted for sex, age, and body mass index or fat mass (%).
food intake (9, 27). It is a strength of our study design that couples that have shared a large part of their adult environment and major lifestyle factors were enrolled. Although it is impossible to completely eliminate the effect of these confounding factors, the opportunity to use a pairwise comparison has enabled us to minimize their effects. Although the sex distribution tended to be unequal between groups of offspring and controls, the IMCL levels between men and women did not differ significantly.

In a previous study, we found a 25% increase in peripheral insulin sensitivity, but not hepatic insulin sensitivity, for offspring compared with controls, as measured with a hyperinsulinemic euglycemic clamp study, a difference that could not be explained by differences in body composition (33). In the present study we found that, despite similar body composition, offspring had lower IMCL levels compared with controls. In previous studies, differences in IMCL levels were observed between groups of insulin-sensitive and insulin-resistant subjects and in some but not all studies between nondiabetic and diabetic subjects (2, 11, 15, 17, 18, 26). Moreover, in lean young subjects with normal glucose tolerance, differences in IMCL levels were found to associate specifically with differences in peripheral but not hepatic insulin sensitivity (22).

IMCL corresponds to small intramuscular lipid droplets that are located close to the mitochondria, where they serve as a rapidly available energy source for muscular fatty acid oxidation. In support of this physiological role, when the need for muscular fatty acid oxidation is increased, such as upon endurance training, IMCL levels are increased concordantly (27, 28). This may explain the observation of higher IMCL levels in marathon runners who are insulin sensitive and have a high mitochondrial content (6). However, increased IMCL levels may be associated with decreased insulin sensitivity when the supply of fatty acids to the muscle exceeds the capacity for muscular fat oxidation, because accumulated fatty acids and their derivatives in IMCL may interfere with muscle insulin signaling (16). Such a mismatch between the supply and oxidation of fatty acids can be induced by a positive energy balance (due to overnutrition, physical inactivity, or any combination between the two) or a reduced capacity for muscular fat oxidation (i.e., reduced mitochondrial capacity). It is a drawback of our study that we have not measured mitochondrial function and/or numbers, and therefore, we cannot be certain as to what mechanisms may have resulted in the lower IMCL levels observed in offspring. Because offspring-partner couples were matched for major lifestyle factors, including physical activity, we speculate that the lower IMCL levels found in the offspring might be a result of better mitochondrial capacity in this population. Alternatively, or in addition, high IMCL levels can also be the cause of mitochondrial dysfunction (reviewed in Ref. 25). Previous research has shown that elderly subjects have a decreased mitochondrial function and higher IMCL levels compared with young subjects (17). Our earlier observations showed that offspring have a lower prevalence of age-related disease, suggesting that they are biologically younger than controls. This may explain the lower IMCL levels found in this study group.

Another drawback of our study was that we did not reassess insulin sensitivity in this study population, and therefore, we cannot quantitatively relate the difference that was observed in IMCL levels to the difference in insulin sensitivity between groups. However, available data from different subgroups do indicate that differences in both insulin sensitivity and fasted glucose levels were present between (sub)groups, and we found that fasted glucose levels were positively associated with IMCL levels. Moreover, the observed differences in insulin sensitivity and fasted glucose levels in the subgroups suggest that in the present study cohort, in which the exclusion criteria for the MRS measurements were numerous but less strict with respect to metabolic health criteria, the differences in insulin sensitivity may have been larger than the 25% that we observed previously. This is strengthened by the fact that we found 50% lower IMCL levels in the offspring group compared with their partners who would have, based on earlier studies, corresponded with an approximate difference of 50% in insulin sensitivity (11, 15, 18). Speculating, we think that the difference found in IMCL levels is striking, and our results suggest that the mechanisms involved in IMCL levels could provide an explanation for the enhanced insulin sensitivity found in subjects predisposed to longevity.

The relationship between insulin sensitivity and IMCL content may differ depending on muscle type and compartment. The tibialis anterior muscle is a predominantly glycolytic muscle and shows lower IMCL content than, for example, the soleus muscle (10). This could have resulted in lower sensitivity to detect differences in IMCL between groups in this specific muscle, thus underestimating the potential difference existing in a more oxidative muscle.

The strength of our study is that for our data acquisition we used high-field 7 Tesla MRI in the tibialis anterior muscle. Earlier studies using 1.5 and 3 Tesla have a relatively lower spectral dispersion, which could lead to an overestimation of IMCL levels because of contamination of the signal from overlapping EMCL resonances (1). In the present study, the spectral separation between EMCL and IMCL was maximized by studying the tibialis anterior muscle due to the fiber alignment being parallel to the magnetic field. All spectra acquired in our study showed distinct IMCL and EMCL peaks, which strengthens the reliability of our results.

In conclusion, the offspring of nonagenarian siblings predisposed to longevity have lower IMCL levels in the tibialis anterior muscle compared with their partners, as measured with short echo time \(^1\)H-MRS at a field strength of 7 Tesla. Further research using \(^31\)P-MRS in combination with \(^1\)H-MRS to determine the phosphocreatine recovery of the muscle could be performed to assess the in vivo mitochondrial capacity in our study population. This could provide more insight into the mechanism behind the lower IMCL levels and their association with insulin sensitivity.

ACKNOWLEDGMENTS

We thank all of the study participants for their contribution.

GRANTS

The study was supported by the Innovation Oriented Research Program on Genomics (SenterNovem; IGE01014 and IGE5007) and The Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (NGI/ NWO: 05040202 and 050-060-810).

DISCLOSURES

The authors have no conflicts of interest.
AUTHOR CONTRIBUTIONS

C.W., A.M., R.W., E.S., S.M., and D.v.H. did the conception and design of the research; C.W., A.v.O., H.K., and A.W. performed the experiments; C.W. and A.v.O. analyzed the data; C.W., A.v.O., H.K., A.M., and D.v.H. interpreted the results of the experiments; C.W. and A.v.O. prepared the figures; C.W., A.v.O., and D.v.H. drafted the manuscript; C.W., A.v.O., H.K., A.M., R.W., E.S., A.W., S.M., and D.v.H. approved the final version of the manuscript; H.K., A.M., R.W., E.S., A.W., S.M., and D.v.H. edited and revised the manuscript.

REFERENCES