Insulin Resistance and Impaired Mitochondrial Function in Obese Adolescent Girls

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Abstract

Background: Mitochondrial dysfunction plays a role in the development of muscle insulin resistance (IR) and the accumulation of intramyocellular lipid (IMCL) in skeletal muscle that can, in turn, interfere with insulin signaling. The purpose of this study was to assess mitochondrial function (MF) and IMCL in obese adolescent girls with and without IR to determine whether: (1) Girls with IR have impaired MF, and (2) impaired MF in girls with IR is related to higher IMCL.

Methods: We examined 22 obese girls aged 13–21 years old for IR [defined as a homeostasis model assessment of insulin resistance (HOMA-IR) value >4]. Phosphorus magnetic resonance spectroscopy (31P-MRS) and proton magnetic resonance spectroscopy (1H-MRS), respectively, were used to determine MF and IMCL of the soleus muscle along with magnetic resonance imaging (MRI) measures of visceral, subcutaneous, and total adipose tissue (VAT, SAT, and TAT) in girls with HOMA-IR >4 (insulin-resistant group) versus HOMA-IR ≤4 (insulin-sensitive group). Serum lipids and waist-to-hip ratio (W/H) were also measured.

Results: Girls with IR ($n=8$) did not differ from the insulin-sensitive group ($n=14$) for age, bone age, weight, VAT, SAT, TAT, or IMCL. However, the insulin-resistant group had higher W/H. Additionally the insulin-resistance group had a lower log rate of postexercise phosphocreatine (PCr) recovery (ViPCr) and a higher log PCr recovery constant (τ), indicative of impaired MF.

Conclusions: Obese girls with increased IR have impaired mitochondrial function. This association is not mediated by alterations in IMCL or adipose tissue. Further studies are necessary to determine whether there is a causal relation between impaired mitochondrial function and IR in obesity and mediators of such a relationship.

Introduction

OBESITY IS A GLOBAL PHENOMENON, and the United States is leading the upward trend in obesity rates in industrialized countries.¹ The prevalence of obesity has tripled among adolescents aged 12–19 years over the last two decades to approximately 17%.^{2–4} Of particular concern, 84–90% of overweight adolescents will become obese adults,^{5,6} and obesity that persists into adulthood increases the risks of cardiovascular disease and diabetes.⁷ In fact, obesity is the most common cause of insulin resistance (IR)^{8,9} and type 2 diabetes (T2D)^{7–9} in children. Along with, and related to the increase in obesity rates, the prevalence of T2D is increasing in epidemic proportions.^{10,11} To develop the most effective therapeutic strategies to treat IR and T2D, it is imperative to first comprehensively understand the determinants of IR in

obesity. Additionally, because IR peaks in adolescence consequent to specific hormonal changes related to puberty,¹⁰ it is not clear that data from adults can be extrapolated to obese adolescents.

Skeletal muscle is the most important site for insulin-mediated glucose uptake¹¹ and also one of the earliest detectable locations showing signs of IR in obesity.⁹ Muscle IR is accompanied by an increase in intramyocellular lipid (IMCL) content when compared to insulin-sensitive counterparts,^{12–14} and accumulation of lipid metabolites in muscle has been implicated as an etiologic factor in the development of muscle IR.^{9,11} Recent data suggest that mitochondrial dysfunction is also correlated with the development of muscle IR.^{12,15,16} Mitochondrial dysfunction resulting in decreased oxidative capacity^{14,17} can cause accumulation of IMCL,^{9,14} which then interferes with insulin

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signaling.¹¹ Mitochondrial function and IMCL can be assessed noninvasively using phosphorus magnetic resonance spectroscopy (31P-MRS)¹⁸ and proton magnetic resonance spectroscopy (1H-MRS),^{18,19} respectively. There are limited data regarding the relationship between mitochondrial function, IMCL, and IR in obese adolescents. We hypothesized that obese adolescent females with insulin IR [defined as a homeostasis model assessment of insulin resistance (HOMA-IR) >4 ²⁰ would have impaired mitochondrial function associated with increased IMCL compared to obese adolescent females who are not insulin resistant (HOMA-IR ≤ 4). To test this hypothesis, we determined mitochondrial function and IMCL using MRS, along with measures of regional fat using magnetic resonance imaging (MRI) and fasting lipids in obese insulin-resistant compared to obese insulin-sensitive girls.

Methods and Procedures

The study was approved by the Institutional Review Board of Massachusetts General Hospital and Partners HealthCare system. Written informed consent or parental consent with participant assent was obtained from all participants.

Subjects

Participants were recruited through area medical and obesity clinics and advertisements. Thirty-two girls and young women 13–21 years old were screened for study eligibility. Of the screened participants, 22 obese adolescents met inclusion criteria and completed the study visit. Inclusion criteria comprised: (1) Bone age of at least 14 years, (2) body mass index (BMI) greater than the 95th percentile for age (based on the 2000 Centers for Disease Control and Prevention Growth Charts),²¹ or greater than 30 kg/m² if age >18 years, (3) abdominal obesity with a waist-to-hip ratio (W/H) >0.85 , and (4) stable weight (defined as <5 kg change in weight in the prior 3 months).

We limited our study to girls with a bone age of at least 14 years (who would be expected to have attained near-adult height) to reduce the confounding effects of changing growth hormone levels, which would otherwise impact IR in puberty. We also only included girls with high W/H ratios, to maximize our yield of girls with evidence of IR. Exclusion criteria included pregnancy or breast-feeding, history of diabetes mellitus, untreated thyroid dysfunction, renal insufficiency, past or current history of cancer, genetic syndromes causing obesity, and the use of medications known to alter glucose metabolism or body composition (oral contraceptive pills, long-term glucocorticoids, metformin, sibutramine, and orlistat). Additionally, we excluded girls who had new (<6 months) or unstable dosing (dosage change within 3 months) of antipsychotic medication that can cause weight gain (such as olanzapine, clozapine, and risperdone).

Study procedures

Anthropometric measurements. The study was conducted at the Clinical Research Center of the Massachusetts General Hospital. Height was measured as the average of three measurements to the nearest 0.1 cm on a calibrated single wall-mounted stadiometer. Participants, wearing a hospital gown, were weighed to the nearest 0.1 kg on a calibrated electronic scale. BMI was calculated as weight (in kg) divided by height (in meters²). Because BMI varies according to age,

we standardized the value for age with the use of the 2000 CDC charts and conversion to a Z-score. Waist measurements were taken with a plastic tape measure to the nearest 0.1 cm at the level of the iliac crest and umbilicus; the maximum hip circumference was also measured. All measurements were taken at the end of expiration with the subject standing. W/H ratio was calculated as the iliac divided by the hip circumference measurement. Bone age was assessed using methods of Greulich and Pyle.²² The stage of puberty was also determined during the physical examination according to the criteria of Tanner.²³

Experimental protocol. During the study visit at the Clinical Research Center (CRC) of the Massachusetts General Hospital, each subject underwent a complete history and physical examination. Overnight fasting screening laboratory results (thyroid-stimulating hormone, comprehensive metabolic panel, liver function tests, and a complete blood count) were obtained. A 2-h oral glucose tolerance test (OGTT) was performed using a 1.75 grams/kg glucose load (maximum 75 grams). One participant had impaired glucose tolerance (2-h glucose of 141 mg/dL following a 75-gram oral glucose load), and no subject had diabetes.

Abdominal fat depots were quantified using a 3.0 T MRI system (Siemens Trio; Siemens Medical Systems, Erlangen, Germany). A single-slice MRI through the abdomen at the level of L4 was obtained as previously described²⁴ and visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and total adipose tissue (TAT, the sum of VAT and SAT) areas were determined based on offline analysis of tracings obtained using commercial software (VITRAK, Merge/eFilm, Milwaukee, WI). 1H-MRS of the calf was performed to assess IMCL. For the calf muscle 1H-MRS, a voxel measuring 15 $\times$ 15 $\times$ 15 mm (3.4 mL) was placed on axial T1-weighted slice with the largest muscle cross-sectional area of the soleus muscle (S-IMCL), avoiding visible interstitial tissue, fat, or vessels. Single-voxel 1H-MRS data were acquired using point-resolved spatially localized spectroscopy pulse sequence as previously described.²⁵ Fitting of all 1H-MRS data was performed using LCModel (version 6.1-4A; Stephen Provencher, Oakville, Ontario, Canada) as previously described.²⁵ Data for IMCL (1.3 ppm) and extramyocellular lipids (EMCL) (1.5 ppm) methylene protons were used for statistical analyses. LCModel IMCL and EMCL estimates were automatically scaled to unsuppressed water peak and expressed as lipid-to-water ratio.

Mitochondrial function was assessed by dynamic 31P-MRS on the same 3T MRI using an MR-compatible lower leg exercise device²⁶ for investigation of *in vivo* mitochondrial function. For 31P-MRS, a custom-built single-tuned 31P surface coil was placed in contact with posterior calf muscles. Initially, 31P spectra were acquired from the resting muscles under fully relaxed conditions (TR 15,000 msec, TE 0.225 msec, 16 averages, bandwidth 2000 Hz, flip angle 90°). Maximum voluntary contraction (MVC) for plantar flexion was determined before the exercise protocol based on maximum force applied against a handheld dynamometer (μ Fet2, Hoggan Health Industries, West Jordan, UT). The exercise protocol consisted of 2 min of rest (60 acquisitions), followed by 3 min plantar flexion against a load of 30% MVC (90 acquisitions) at a constant frequency of 0.5 Hz (every 2 sec), followed by 5 min of recovery (150 acquisitions). Phosphocreatine (PCr), inorganic phosphate (Pi), and adenosine triphosphate (ATP) resonances were fitted using in-house

software developed in MATLAB (The Mathworks, Inc., Natick, MA). Mitochondrial function was determined by plotting the PCr peak integrated area versus time during exercise recovery and fitting recovery curves to a monoexponential function to determine the PCr recovery time constant tau, a marker of mitochondrial dysfunction, and ViPCr (1/tau normalized for PCr depletion), a marker of mitochondrial function. All body composition analyses were performed by study personnel blinded to the randomization assignment. MRI/MRS data were available in 19 participants.

Biochemical assessment. Fasting insulin levels were analyzed by immunoassay [Cobas, Roche Diagnostics, Indianapolis, IN; lowest detectable concentration 0.2 μ U/mL, coefficient of variation (CV) 0.8–3.7%]. Glucose was analyzed via an enzymatic *in vitro* test (Cobas, Roche Diagnostics, Indianapolis, IN; lowest detectable concentration 2 mg/dL, intra-assay CV 1.0%). Total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were analyzed via a Roche direct assay (Cobas, Roche Diagnostics, Indianapolis, IN); total cholesterol (lowest detectable concentration 3 mg/dL, CV 0.8–1.0%), LDL (lowest detectable concentration 3 mg/dL, intra-assay CV 0.71–1.22%), and HDL (lowest detection limit 3 mg/dL, intra-assay CV 0.60–0.95%). Triglycerides were analyzed via the Roche triglyceride assay (Cobas, Roche Diagnostics, Indianapolis, IN); triglyceride (lowest detectable concentration 4 mg/dL, intra-assay CV 0.9–1.5%). Very-low-density lipoprotein (VLDL) was calculated by subtracting both LDL and HDL from total cholesterol. Fasting insulin was analyzed in one single batch sample from serum that was stored at -80°C until analysis. All other samples were analyzed immediately.

IR was measured by homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR was calculated as the product of the fasting plasma insulin level (in microunits per milliliter) and the fasting plasma glucose level in millimoles per liter, divided by 22.5 $\{[\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{U/mL)}] / 22.5\}$.^{20,27,28}

Statistical methods. JMP Statistical Discoveries (version 10; SAS Institute, Inc., Cary, NC) was used for all analyses. Data are reported as means $\pm$ standard deviation (SD), unless otherwise indicated. Obese girls were divided into insulin-resistant or insulin-sensitive groups based on a HOMA-IR value of >4 or ≤ 4 , respectively.²⁰ We also analyzed our data by dichotomizing study subjects based on the median HOMA for the group (median 3.35). We used the Student's *t*-test to compare differences between groups when data were normally distributed, or when a logarithmic transformation led to a normal distribution. Parametric (Pearson) or nonparametric (Spearman) correlations were used as appropriate to determine associations between variables that were or were not normally distributed. Significance was defined as a two-tailed $P < 0.05$.

Results

Subject characteristics

Clinical characteristics of our participants are summarized in Table 1. The study participants had a mean age of 16.6 ± 2.3 years, a mean BMI of $38.5 \pm 7.5 \text{ kg/m}^2$, and a mean BMI standard deviation score (SDS) of 2.2 ± 0.4 . The insulin-resistant group (HOMA >4) ($n=8$) did not differ for age or bone age from the insulin-sensitive (HOMA ≤ 4) ($n=14$) group. In addition, weight, BMI, and BMI SDS did not differ between groups.

TABLE 1. CLINICAL CHARACTERISTICS OF OBESE ADOLESCENT GIRLS CHARACTERIZED AS INSULIN RESISTANT VERSUS INSULIN SENSITIVE BASED ON A HOMA-IR VALUE >4 OR ≤ 4

	Insulin resistant (HOMA-IR >4) ($n=8$)	Insulin sensitive (HOMA-IR ≤ 4) ($n=14$)	P
Age (years)	15.6 ± 2.1	17.2 ± 2.3	0.12
Bone age (years)	16.8 ± 1.3	17.3 ± 1.2	0.28 ^a
Weight (kg)	109.0 ± 20.5	96.5 ± 19.8	0.17
BMI (kg/m^2)	41.2 ± 8.9	37.0 ± 6.5	0.22
BMI SDS	2.4 ± 0.5	2.1 ± 0.4	0.17
SBP (mmHg)	116.6 ± 8.6	112.7 ± 8.7	0.32
DBP (mmHg)	72.1 ± 10.2	74.1 ± 5.9	0.56

^aWilcoxon test.

HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index; BMI SDS, body mass index standard deviations; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Participants or their parents provided information about race and ethnicity; 6 participants were white (27.3%), 4 were black (18.2 %), 7 were Hispanic (31.8%), 1 was Native American (4.6%), 1 was Asian (4.6%), and 3 classified themselves as unknown or of more than 1 race (13.6%).

Body composition and IMCL

Body composition and IMCL results are summarized in Table 2. The insulin-resistant group had an increased W/H ratio ($P=0.002$) and trended to have increased umbilicus circumference ($P=0.08$) compared to the insulin-sensitive group. However, the groups did not differ for IMCL of soleus (Fig. 1A). Likewise there was no difference between the groups for SAT, VAT, or TAT.

Mitochondrial function

The insulin-resistant group had lower log ViPCr, a marker of initial rate of postexercise PCr recovery ($P=0.04$) (Table 2, Fig. 1B), and higher log tau, a marker of overall mitochondrial dysfunction and PCr recovery rate ($P=0.05$) compared to the insulin-sensitive group (Table 2), indicative of impaired mitochondrial function in obese adolescent girls with IR. Similar results were seen when insulin-resistant and insulin-sensitive groups were dichotomized by HOMA-IR above or below the median (3.35) [log ViPCr (2.0 ± 0.8 vs. $2.9 \pm 0.6 \text{ mmol/min}$; $P=0.02$). There was a negative correlation between ViPCr and HOMA-IR (Spearman $\rho = -0.45$, $P=0.05$).

Serum lipids

When the group was dichotomized by HOMA-IR above or below our study median (3.35), the insulin-resistant group had lower HDL (40.6 ± 5.6 vs. $47.8 \pm 10.1 \text{ mg/dL}$; $P=0.049$) and higher VLDL (23.3 ± 7.5 vs. $15.5 \pm 7.9 \text{ mg/dL}$; $P=0.03$) and triglycerides (116.4 ± 37.4 vs. $77.2 \pm 39.7 \text{ mg/dL}$; $P=0.03$) than the insulin-sensitive group. There were no differences between groups for total cholesterol or LDL. We found no relationship between mitochondrial function and lipid levels.

Discussion

Our data show that within a group of obese adolescent girls, IR (assessed by HOMA-IR) is associated with

TABLE 2. BODY COMPOSITION, IMCL, MITOCHONDRIAL FUNCTION, AND LIPIDS IN OBESE ADOLESCENT GIRLS CHARACTERIZED AS INSULIN RESISTANT VERSUS INSULIN SENSITIVE BASED ON A HOMA-IR VALUE >4 OR ≤ 4

	Insulin resistant (HOMA-IR >4) (n=8)	Insulin sensitive (HOMA-IR ≤ 4) (n=14)	P
W/H ratio	0.99 $\pm$ 0.06	0.91 $\pm$ 0.05	0.002
Umbilicus (cm)	124.9 $\pm$ 15.8	112.8 $\pm$ 14.5	0.08
SAT (mm ²)	67,244 $\pm$ 19494	55,569 $\pm$ 13793	0.13
TAT (mm ²)	77,318 $\pm$ 19748	64,136 $\pm$ 16056	0.12
VAT(mm ²)	10,074 $\pm$ 3113	8567 $\pm$ 3573	0.35
Log ViPCr (mmol/min)	1.89 $\pm$ 0.95	2.72 $\pm$ 0.59	0.04
Log tau (s)	3.84 $\pm$ 0.56	3.46 $\pm$ 0.24	0.0507^a
Log S-IMCL/W	-3.92 $\pm$ 0.58	-3.53 $\pm$ 0.50	0.12
TA-IMCL/W	0.0050 $\pm$ 0.003	0.0045 $\pm$ 0.001	0.54
Total cholesterol (mg/dL)	157.3 $\pm$ 27.6	164.4 $\pm$ 40.2	0.66
LDL (mg/dL)	92.5 $\pm$ 25.8	101.5 $\pm$ 31.7	0.50
HDL (mg/dL)	42.0 $\pm$ 5.1	45.4 $\pm$ 10.3	0.39
VLDL (mg/dL)	22.8 $\pm$ 7.8	17.4 $\pm$ 8.6	0.16
Triglyceride (mg/dL)	113.5 $\pm$ 38.9	87.2 $\pm$ 43.1	0.17

Statistically significant figures are in bold.

^aP=0.09 with the Wilcoxon test.

IMCL, intramyocellular lipid; HOMA-IR, homeostasis model assessment of insulin resistance; W/H ratio, waist-to-hip ratio; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue; ViPCr, 1/tau normalized for phosphocreatine depletion; tau, phosphocreatine recovery time constant; S-IMCL/W, TA-IMCL/W, soleus, tibialis anterior intramyocellular lipid ratioed for water; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein.

FIG. 1. Intramyocellular lipids (IMCL) and mitochondrial function in insulin-resistant versus insulin-sensitive obese adolescents. (A) There was no significant difference in IMCL levels between the two groups ($P=0.12$). (B) Log ViPCr (initial rate of postexercise PCr recovery) was lower in insulin-resistant versus insulin-sensitive obese adolescent girls ($P=0.04$). SOL, soleus; HOMA-IR, homeostasis model assessment of insulin resistance.

mitochondrial dysfunction and abnormal serum lipids, but not with differences in fat mass or IMCL compared to age and BMI-matched insulin-sensitive girls. Although mitochondrial function and IMCL have been studied in insulin-resistant adults,^{12,14,29} limited studies have examined the role of mitochondrial dysfunction and IMCL as potential mediators of insulin resistance in obese adolescents.

It is well known that adolescents experience a period of increased IR as they progress through puberty.¹⁰ Because a HOMA-IR value >4 is considered a cutoff value for IR in adolescents,²⁰ we chose to dichotomize our group into insulin-resistant or insulin-sensitive groups based on a HOMA-IR value of >4 or ≤ 4 . However, a HOMA-IR of >3.16 has also been reported to be the upper range for normal insulin sensitivity in children and adolescents³⁰; therefore, our study median of 3.35 for HOMA-IR also provides an accurate, generalizable, if slightly less conservative, cutoff for differentiating between insulin-resistant and insulin-sensitive subjects.

Being overweight or obese are among the most common causes of IR and T2D^{7-9, 31}; an overweight diagnosis (BMI ≥ 25) was attributed to 61% of the cases of T2D diagnosed in adult females in a 16-year follow-up study.³¹ Because skeletal muscle is the main target for insulin-mediated glucose disposal,^{11,32} it is also one of the earliest detectable locations to show signs of IR in obesity.⁹ IMCL has been identified to mediate the development of IR in obesity.¹¹ To this end, a previous study showed that insulin sensitivity was inversely correlated with IMCL in sedentary middle-aged adults.¹³ In that same study, however, adult endurance athletes had higher IMCL content, similar to those with T2D, despite greater insulin sensitivity than their obese counterparts, with IMCL likely representing an energy source for muscle contraction.³³ Lipid metabolites¹³ such as diacylglycerol (DAG)³⁴ and ceramide,^{11,34} rather than IMCL alone, are likely responsible for insulin-stimulated glucose metabolism.¹³ Previously, we have reported associations of IMCL with HOMA-IR in a combined group of obese and normal-

weight adolescent girls.³⁵ However, in our current study of obese adolescent subjects with a W/H ratio >0.85 , IMCL content did not differentiate between girls who did or did not have IR, suggesting that within an obese adolescent population, IMCL may not be a reliable marker of IR.

Mitochondrial dysfunction has been implicated in the development of muscle IR. Mitochondria, abundant in skeletal muscle, are the major functional components of cellular fuel oxidation and ATP production.^{16,36} In both obesity and T2D, skeletal muscle mitochondria are smaller and occasionally damaged,¹⁶ and altered mitochondrial morphology correlates with the degree of IR.^{16,36} However, the morphology of mitochondria can only be determined microscopically after a biopsy.³⁶ In this regard, dynamic ³¹P-MRS is an excellent noninvasive technique to assess mitochondrial function. Mitochondrial function predisposes adults to IMCL accumulation,⁹ and IMCL accumulation may lead to IR.^{9,33} Given that IR peaks during adolescence, this period provides a unique opportunity (1) to study the association of mitochondrial function with IMCL and IR, and (2) for intervention that may prevent long-term health consequences in obesity.

Our study suggests that IR in adolescent girls is related to mitochondrial dysfunction as assessed by PCr recovery kinetics. However, we did not find this relationship to be mediated by IMCL, SAT, or VAT, which have been shown to be related to IR in other studies.^{9,33,37} These findings led us to infer that in adolescence, mitochondrial dysfunction may be a very early indicator of IR and may precede changes in IMCL, VAT, or SAT. The ability to safely and noninvasively screen for mitochondrial dysfunction provides possibly the earliest imaging strategy to identify adolescents with risk of developing IR. Targeting these adolescents, who then may be at particular risk for developing diabetes and other long-term health consequences, could allow for a time of maximal impact in the prevention of numerous serious co-morbidities. Importantly, even within girls with a W/H ratio of >0.85 , the W/H ratio did differentiate between obese girls with or without IR, suggesting that this clinical measurement is a powerful determinant of IR states in obese adolescents.

Limitations of our study include its small size and its associative and cross-sectional nature. Inclusion of more insulin-resistant subjects (when the group was dichotomized by HOMA-IR values above or below 4) may have further strengthened our results. However, a significant strength is that we used state-of-the-art, noninvasive imaging and spectroscopic techniques to assess the impact of mitochondrial function, IMCL, and regional fat deposition on IR in a young obese population. In addition, it would have been optimal to also assess hepatic lipid deposition as a measure of IR in our subjects in relation to mitochondrial function, and we plan to examine associations of mitochondrial function with hepatic fat in future studies. Furthermore, it is possible that limiting our enrollment to girls with a W/H ratio of >0.85 limited the variability of our end points and our ability to detect significant differences in certain parameters. Further studies are necessary to determine whether there is a causal relation between impaired mitochondrial function and IR in obesity and mediators of such a relationship. Finally, our data confirm an association between IR and an unfavorable lipid profile, indicating that without intervention, these girls may be at significant risk for cardiovascular complications.

In conclusion, our study suggests that mitochondrial function (as assessed with ³¹P-MRS) is impaired in obese

adolescents with IR despite similar IMCL levels compared to obese insulin-sensitive girls. This suggests that mitochondrial dysfunction may play an etiologic role in the development of IR before changes in IMCL are detectable. Further studies are necessary to confirm these findings.

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M.M. conceived the study; M.B., M.S., and M.M. carried out study-related procedures and performed data collection; M.S., M.B., M.T., and M.M. analyzed and interpreted data; H.T. worked on data collection and analysis; M.S. performed the literature search; M.S., M.B., and M.M. wrote the manuscript and generated the figures; M.M. had primary responsibility for final content. All authors were involved in manuscript preparation and approved the submitted version.

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Author Disclosure Statement

No competing financial interests exist.

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