The Effect of Exercise on Intramyocellular and Extramyocellular Lipids in Childhood Versus Adulthood Onset of Obesity: A Pilot Study

Sarah Feola

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By: Sarah Feola

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Signed by the final examining committee:

Dr. Robert Kilgour Chair

Dr. Maryse Fortin Examiner

Dr. Jose Morais Examiner

Dr Sylvia Santosa Thesis Supervisor

Approved by Dr. Geoffrey Dover, Graduate Program Director

Dr. Pascale Sicotte, Dean of Faculty

Abstract **Sarah Feola**

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Background: Compared to those who develop obesity in adulthood, those with childhood onset obesity have greater risk of metabolic disease. However, the mechanisms associated with this increased risk are unknown. Muscle lipids have been associated with insulin resistance. Thus, differences in the metabolism of intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) may contribute to greater risk of metabolic disease in childhood vs. adulthood onset obesity.

Objective: To investigate whether the timing of obesity onset affected the metabolism of IMCL and EMCL in response to exercise in individuals with obesity.

Methods: Ten males with obesity ≥23yrs were recruited with either childhood- (n=5) or adulthood- onset (n=5) obesity. At visit 1, participants underwent dual x-ray absorptiometry (DXA) and resting indirect calorimetry. Participants were provided with a standardized diet for 3 days prior to visit 2. At visit 2, baseline IMCL and EMCL via magnetic resonance spectroscopy (MRS) was measured. Participants then completed a 90 minute cycle with indirect calorimetry. A second MRS measurement was done immediately post exercise.

Results: Substrate oxidation was not significant between groups. Post exercise, the adulthood onset group had a significant (P=0.039) decrease in IMCL, with no change in EMCL. The childhood onset group displayed no change in IMCL or EMCL with exercise.

Conclusion: This was the first study to compare the rates of IMCL and EMCL depletion in childhood and adulthood onset of obesity after a bout of exercise, providing novel insight for future research. We made a novel observation that males with adulthood onset obesity had greater decreases in IMCL post-exercise.

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Contribution of Authors

As the primary author, I was responsible for the recruitment of participants, protocols, methodology, statistical analyses and the written literature. As the principal investigator, Dr. Santosa was responsible for the conceptualization, funding acquisition, and revision of the study presented in this thesis.

Table of contents

List of figures……viii

List of table……..……..ix

Abbreviation……x

1. Background and literature review…….……1

1.1 Prevalence of obesity……..1

1.2 The age of obesity onset as a risk factor for obesity associated-comorbidities……..……1

1.3 What is IMCL & EMCL…….……3

1.4 IMCL & EMCL use during exercise……...……6

1.5 Ways to measure IMCL and EMCL……...……..10

2. Rationale…….……...11

3. Objective……12

4.Hypothesis ……..……12

5. Methods……..……12

5.1 Participants…….…….……12

5.2 Study visit…….……13

5.3 Body composition……...……..13

5.4 Diet intervention…….……..……14

5.5 Substrate oxidation via indirect calorimetry at rest and during exercise…….……..14

5.6 Exercise protocol……..……15

5.7 Magnetic resonance spectroscopy……..…….…….15

5.8 Statistical analysis…….……..…….17

6. Results……18

6.1 Characteristics of participants…….……..18

6.2 Resting energy expenditure and resting substrate oxidation…….……18

6.3 Substrate oxidation during exercise…….…….19

6.4 Intramyocellular lipids and extramyocellular lipids ……...……..19

6.5 Relationships……...……..……...21

7. Discussion……...……22

7.1 Energy expenditure and substrate oxidation……...…..…….22

7.2 IMCL & EMCL……...…….22

7.3 Strengths and limitations……..……24

7.4 Strengths……..…….……24

7.5 Limitations……...……24

8. Conclusion……..……..……..25

9. References……..26

List of Figures

Figure 1: MRS voxel placement ……...…….……17

Figure 2: IMCL & EMCL changes within groups…….…..……20

List of Tables

Table 1: IMCL & EMCL use during a bout of exercise within different participants……..……9

Table 2: Skeletal muscle lipid quantification methods…….……..11

Table 3: Participant characteristics…….……18

Table 4: Resting energy expenditure and substrate oxidation at rest…….……19

Table 5: Substrate oxidation during exercise…….…..……19

Table 6. Combined group IMCL & EMCL……20

Table 7. IMCL & EMCL relationship to fat oxidation at rest, during exercise, total fat and fat mass……21

Abbreviation

A.U. arbitrary units

BMI body mass index

EM electron microscopy

EMCL extramyocellular lipid

FM full marathon

HM half marathon

IMCL intramyocellular lipid

MET metabolic equivalent of task

MRS magnetic resonance spectroscopy

NCR non-competitive runners

REE resting energy expenditure

RER respiratory exchange ratio

SD standard deviation

1. Background and literature review

1.1 Prevalence of obesity

Obesity is a chronic disease resulting from the buildup of excess body fat. Obesity results in increases the risk of illnesses, long-term health complications, and a reduced lifespan [1]. From 1980 to 2013, 2.1 billion people were categorized as overweight or obese, increasing overweight and obesity rates 27.5% amongst adults and 47.1% amongst children [2]. The lack of understanding regarding this epidemic is alarming, considering 340 million children and teenagers aged 5-19 were overweight or obese in 2016, and three-quarters of children will carry their weight into adulthood [3, 4].

1.2 The age of obesity onset as a risk factor for obesity associated-comorbidities

Compared to those who experience the onset of obesity in adulthood, childhood onset of obesity carries an increased risk of type 2 diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer [5, 6] . For example, a study by Pacheco et al. [7], looking at the effects of early onset obesity and metabolic syndrome found that a child who had obesity at the age of 5 had a higher risk score of metabolic syndrome at the age of 16 as compared to those who did not have early onset obesity. Another group also found that compared to those who were not previously overweight in childhood, persistent excess weight from childhood to adulthood increased the risk of type 2 diabetes by 12-fold, as compared to 5-fold when onset was in adulthood [6]. Maximova et al. [8] suggested that more than half of youth living with obesity have at least one cardiovascular risk factor and a quarter of them display more than two risk factors. While the risk of obesity-associated comorbidities appears to be different between individuals with either childhood- or adulthood-onset obesity, the underlying mechanisms affecting those differences are unclear.

One potential underlying mechanism associated with the development of obesity-associated comorbidities may stem from perturbations in lipid metabolism. Kim et al. [9] found that obesity factored in the inability to properly oxidize lipids. Once obesity onset took place (BMI $\geq$30kg/m^2) lipid oxidation decreased, though lipid oxidation was not further influenced by obesity classes [9]. Kelley et al. [10], also found that both before and after weight loss, there was a lower rate of fat oxidation within the thigh of individuals with obesity. The reasoning behind this phenomenon has yet to be understood [9].

Studies published so far have shown fundamental difference between those with childhood- and adulthood- onset obesity that likely contribute to the differences observed in disease risk. A study from our lab looking at childhood vs. adulthood onset obesity saw a reduced amount of acetyl-CoA in those with childhood vs. adulthood onset obesity indicating potential differences substrate metabolism [11]. Additionally, a study by Rupp et al., [12] looking at childhood vs. adulthood onset obesity found that the childhood onset group had significantly higher cardiorespiratory fitness. However, it should be noted that the age of the groups were not matched [12]. Previous literature looking at adipose tissue in those with childhood onset obesity showed that expansion occurred as a result of hyperplasia, whereas in the adulthood onset groups adipose tissue expansion was by hypertrophy [13, 14]. These differences suggests that age of obesity onset may affect adipose tissue size, and metabolism. The difference in childhood vs. adulthood onset obesity in skeletal muscle metabolism has not yet been understood.

1.3 - What is IMCL and EMCL?

In obesity, increased fat buildup in skeletal muscles can be divided into two categories: intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) [15]. Intramyocellular lipids are lipid droplets stored within the cell of the muscle, whereas extramyocellular lipids are stored interstitially outside the muscle fiber (e.g. fatty streaks in muscle) [15, 16]. Fat stored as IMCL are more dynamic than EMCL and is more readily available for energy production [17]. IMCL can accumulate in muscle due to elevated levels of fatty acids and impaired fat oxidation, often observed in those with obesity and sedentary lifestyles [18]. Individuals with obesity have been observed to have increased fatty acid content in skeletal muscle [19, 20] as well as, the inability to properly oxidize lipids [9].

Both IMCL and EMCL have been investigated in the context of insulin resistance. In comparison to evidence indicating an effect of IMCL on insulin resistance, the relationship between EMCL and insulin resistance has not been well studied [21]. Although EMCL within the soleus muscle has been reported as significantly increased in individuals with obesity [22, 23], the importance of EMCL within muscle metabolism has been widely disregarded [24]. Fisher et al. [25] found a correlation between EMCL to total, and relative body fat in patients with a BMI >40kg/m^2, however, the relationship to insulin was not accounted for. Barrera et al. [26] found a significant correlation between IMCL and insulin resistance, but not with EMCL in men with BMI ≥25kg/m^2. This study also found a significant correlation was found between abdominal and trunk lipid accumulation and EMCL and IMCL [26].

An accumulation of IMCL has been associated with obesity, insulin resistance and the development of type 2 diabetes in adults [27]. Barrera et al. [26] found that both IMCL and EMCL were significantly higher in overweight compared to normal weight participants.

Additionally, insulin sensitivity correlated significantly with IMCL accumulation [26]. Perseghin et al. [28] also saw a significant relationship between IMCL in the soleus and insulin resistance in those with obesity. Hulver et al. [29] reported that IMCL was 22% higher in the vastus lateralis of participants with obesity compared to the normal weight group. Also, IMCL was significantly correlated with increased BMI [29]. Obesity is not only associated with greater IMCL levels but also lower IMCL oxidation. Research comparing 9 lean and 9 females with obesity found that females with obesity had a significantly lower rate of IMCL oxidation [9]. The inability to properly oxidize IMCL can lead to further accumulation. Considering the relationship between IMCL and insulin resistance, it is critical to understand how obesity affects IMCL metabolism. As we do not know the significance of EMCL in the context of insulin resistance and human health, further investigation is needed.

Importantly, individuals with childhood onset obesity are at especially greater risk of insulin resistance and type 2 diabetes [5, 6]. Since IMCL is associated with insulin resistance, understanding IMCL metabolism in individuals with childhood onset obesity may be important to further understand the pathogenesis of obesity associated comorbidities. Although the implication of childhood-onset obesity on IMCL metabolism have never been investigated, greater accumulation of lipids in muscle has been previously observed in children and adolescents with obesity. Even in these younger populations, IMCL is associated with insulin resistance. Sinha et al. [23] found positive associations between EMCL and IMCL and markers of obesity (BMI, percent total body fat) in adolescents. They also found a significant relationship between IMCL and insulin resistance, but the association between EMCL and insulin resistance was weak and not significant [23]. Additionally, Weiss et al. [30] compared 14 insulin sensitive vs 14 insulin resistant adolescents with obesity. Although not all adolescents living with obesity

are insulin resistant nor have increased IMCL levels, those with obesity and who were insulin sensitive had lower intramyocellular lipid deposition compared to those who were insulin resistant [30]. Considering levels of IMCL can play a role in altering insulin function and skeletal muscle lipid metabolism in childhood, it is plausible that individuals with childhood-onset obesity may present aberrant IMCL metabolism.

1.4 Metabolism and substrate oxidation during exercise

Respiratory exchange ratio (RER) is the measurement of CO_2 production relative to O2 consumption. RER is an indicator of the relative contribution of carbohydrate and lipid oxidation to overall energy expenditure. As RER decreases, this indicates the use of lipid oxidation (0.7 maximum lipid oxidation) and as RER increases oxidation shifts towards carbohydrates (1.0 maximum carbohydrate oxidation) [31]. Values ranging from 0.7-1.0 indicate a mixture of both carbohydrates and fats being used as an energy source. Though RER at 0.8 indicates protein oxidation, oxidation of proteins is mostly calculated based on urinary nitrogen [32]. An RER of 1.0 indicates the maximal anaerobic threshold of a person which can take place during maximal exercise nearing exhaustion due to hyperventilation [31]. The value of 0.7 indicates maximal fatty acid oxidation and can occur during prolonged starvation [33]. Due to stoichiometric oxidation equations for substrate oxidation (at which RER would max out at 1.0 for carbohydrate oxidation), RER values exceeding 1.0 are not due to substrate metabolism but rather bicarbonate buffering [34]. A sedentary lifestyle can increase baseline RER, thus indicating decreasing whole body fat oxidation [31].

With exercise, the fuel used to supply working muscle becomes dependent on the intensity and duration at which exercise is performed [35]. As exercise begins, muscles utilize glucose and glycogen to quickly provide energy through anerobic glycolysis [36]. Although

energy is quickly provided through glycolysis, the ATP provided through this anaerobic pathway is insufficient to sustain energy expenditure for long periods of time and results in the accumulation of lactate. In endurance exercise ranging from low to moderate intensity, the formation of pyruvate either enters the mitochondria and is converted to acetyl-CoA or is converted to lactate [36]. Lactate formation occurs when there is an absence of oxygen availability. Lactate can enter the liver where it is converted to pyruvate to form glucose via the Cori cycle [36]. With aerobic exercise, ATP produced by glycolysis predominates as an energy source after 2-3 minutes of exercise, as fatty acids need to be released from fat tissue prior to undergoing β-oxidation, ATP from fat oxidation is slower to become available, glycogen and glucose oxidation continues [35]. As exercise reaches moderate intensities (45-65% VO2max) and a stable state, the availability of oxygen further increases β-oxidation of fatty acids [36]. As fat oxidation rates peak with moderate exercise intensity, IMCL can be broken down and used to supply energy to working muscles [37, 38]. Protein also serves as a source of energy during long duration exercise. Ketogenic and glucogenic amino acids go through deamination (removal of nitrogen) before they can contribute to ATP formation [39]. However, in exercise amino acid oxidation for energy provision is limited to <5% and can rise to 10% during fasted or prolonged exercise [39, 40].

1.4 IMCL and EMCL use during exercise

Our understanding of how IMCL and EMCL contribute to exercise remains incomplete. Factors such as exercise intensity, duration, and fitness level [41-43] can affect the accumulation and breakdown of IMCL. Oppositely, not much is known about the factors affecting EMCL during exercise.

Currently, there are only a few studies examining IMCL and EMCL use in acute bouts of exercise in obesity. Levasseur et al., [44] found no change IMCL (5.4 A.U. to 5.2 A.U., P=0.42) after a 60 minute cycle at moderate intensity in men living with obesity. However, it is likely that 60 minutes of exercise was not enough time to see differences [44]. Gan et al. [45] saw no decreases in IMCL after two 40 minute exercise bouts (55-70% VO2 max) in the soleus (P=0.50) and anterior tibialis (P=0.66) of men living with obesity [45]. Indication of whether exercise was in the fasted state was not mentioned [45]. Whether exercise was done fasted or fed is salient as in the fed state, the release of insulin inhibits lipolysis thus affecting fat oxidation. Though participants were asked not to make dietary changes during the study, the composition of macronutrient intake prior to exercise affects substrate oxidation during exercise [45]. Studies have shown that consumption of a high fat vs high carbohydrate diet shifts oxidation of substrates towards fat or carbohydrates, respectively [42]. With regards to EMCL, Haus et al. [24] reported a decrease in baseline EMCL after 7 days of consecutive aerobic exercise in individuals with obesity. Previous literature has yet to examine the effect of an acute bout of exercise on EMCL in those living with obesity. Collectively, these results indicate that shorter duration exercise may not be sufficient for IMCL or EMCL to be a significant source of energy during exercise [46]. As the balance of macronutrients consumed and whether the exercise is conducted pre- or post-prandial may affect substrate oxidation, further studies should consider these factors.

Though there have only been a few studies on IMCL and EMCL use in acute exercise with obesity; studies in lean individuals may provide some insights. A previous study found that compared to pre-exercise levels, there was a 38% decrease in IMCL following 45 minutes of interval cycling (3 minutes at 50% ventilatory threshold and 2 minutes at 110%) [37]. Another

study by Hinderling et al. [47] reported that after 3-h of cycling at 55% VO_2 max, 8 trained males had a significant decrease in IMCL post cycle, although EMCL did not change. Brechtel et al. [48] assessed the IMCL and EMCL content of 12 male runners, six participated in a leisure run, three in a competitive 21 km and three in a competitive 42 km marathon (diet not controlled for). Post exercise IMCL of the soleus and tibialis anterior decreased in both the leisure runners and 21 km runner who worked at a moderate intensity (60-70% VO_2 max), however, the 42 km runners did not have a decrease in IMCL content while working at >80% VO_2 max [48]. There was no change in EMCL for all three categories of runners [48]. Bucher et al. [49] found a significant decrease in IMCL within 10 lean men who cycled at 50-60% VO_2 max for 2 hours. Considering that less than 60 minutes was not sufficient to measure significant changes in IMCL use in obesity [44, 45], and that changes in lean individuals were observed after longer bouts of exercise, we chose to test the effects of a 90 minute cycle at moderate intensity (40-59% heart rate reserve [50]) in our study.

Table 1. IMCL & EMCL use during a bout of exercise within different participants

Authors	Participants	Protocol	Key measurements/ Method	Location	Major findings
Brechtel, Niess [48]	-Non-competitive runners (NCR) n=6 -Half marathon runners (HM) n=3 -Full marathon runners (FM) n=3	-100 minute run (noncompetitive runners) -21km run -42km run	-IMCL & EMCL -MRS before and immediately after exercise (non-competitive runners) -MRS measured the night before and within one hour after race (full & half marathon runners)	-Tibialis anterior & soleus	-Running at 60-70% of VO2max showed a decrease in post IMCL (NCR & HM group) -The FM group did not show a change in IMCL working at >80%VO2max
Schrauwen-Hinderling, van Loon [47]	-8 highly trained males cyclists	-3-h cycling protocol -Subjects received a standardized diet for 3 days before test	-IMCL & EMCL -MRS before and immediately after exercise	-Vastus lateralis	- ↓IMCL content in vastus lateralis -EMCL content did not change significantly in vastus lateralis
White, Robergs [37]	-9 moderately trained subjects	-45 minutes of interval cycling and 1 hour of recovery -Subjects asked to "maintain similar eating habits" prior to testing day -Three hours before testing 400-kcal meal consumed	-IMCL -MRS measured at rest, immediate post exercise & 60 min into recovery	-Vastus lateralis	-IMCL ↓38% immediately after exercise -IMCL was still ↓30% after 1 h recovery
Décombaz, Fleith [51]	-2 trained men	-2 hour run at moderate intensity (done 3 different times) -Different diets tested after each exercise recovery	-IMCL -Glycogen -H-MRS & C-MRS measured pre-exercise, post-exercise, 10h after & 32h after	-Tibialis anterior	-IMCL & glycogen were both reduced 70% after exercise
Rico-Sanz, Moosavi [52]	-5 healthy male subjects	-90 minute treadmill run @64% VO2 max -Subjects informed to maintain dietary habits	-IMCL -EMCL -MRS pre-exercise, post-exercise measured	-Tibialis anterior & soleus	-IMCL decreased in all subjects soleus and tibialis -IMCL decreased in 4/5 subjects gastrocnemius, but was not significant all together -There were no significant changes in EMCL
Bucher, Krüsi [49]	-10 healthy adult men	-2 hour cycle at 50-60% VO2 max -Diet controlled	-IMCL	-Muscle not specified	-Significant decrease in IMCL (p=0.008) -Insulin sensitivity was not related to IMCL
Ipavec-Levasseur, Croci [53]	-18 obese men	-1h cycling @fatmax -Diet controlled	-IMCL -MRS	-Soleus	-No depletion in IMCL post exercise -No depletion post intervention

1.5 Ways to measure IMCL and EMCL

Fat within skeletal muscles can be quantified in many ways: electron microscopy (EM), Oil red O, and magnetic resonance spectroscopy (MRS). Techniques such as electron microscopy and Oil red O are invasive as they require a biopsy prior to assessment [18, 54] and muscle biopsies have shown variation of up to 23% [55], resulting in error within small changes of the muscle [18]. Once a muscle sample is obtained, EM is ideal to obtain accurate and reproducible measurements. EM only represents a small portion of the muscle and requires many repetitions to allow for complete quantification of muscle IMCL and EMCL [56]. The Oil red O technique requires a biopsy and staining of the muscle prior to being observed under the microscope [18]. However, staining muscle tissue may introduce errors as the oil red O technique stains all neutral lipids interferring with fatty acids [18]. Lastly, magnetic resonance spectroscopy is a non-invasive way to measure chemical compositions of fat depots in humans [57]. It is possible to quantify IMCL and EMCL non-invasively and repeatedly in the same volume of muscle over a large area with an MRS full-body scanner [58, 59]. However, MRS requires precise body and voxel placement, the expertise of trained professionals, availability of an MRI, and is disadvantageous as the technique is expensive to use [59].

Table 2. Skeletal muscle lipid quantification methods

Method	Procedure	Advantage	Disadvantage
Electron microscopy[18, 54-56]	A muscle biopsy, followed by multiple analysis of magnification within small samples of the muscle.	-Accurate -Reproducible	-Small sections of muscle analyzed -Invasive -Time consuming
Oil red O [18, 54, 55]	A muscle biopsy, followed by staining of small sections within the muscle using Oil red O procedure. Analyzed with required imaging system.	-Repeated staining of the same sample	-Invasive -Stains all neutral lipids -Multi-step procedure
Magnetic resonance spectroscopy (MRS)[57-59]	A whole-body magnetic resonance scanner is used with specific placement of a voxel to capture quantity within the muscle.	-Non-invasive -Large sections can be quantified at once -Reproducible	-Expensive -Specific voxel and positioning required for proper analysis -Expertise required

2. Rationale

Individuals with childhood onset obesity are at greater risk of metabolic disease compared to those with adulthood onset obesity. Fundamental differences in adipose tissue characteristics have been observed between these two groups. Several studies have observed greater IMCL and EMCL in adults with obesity [18]. Though the accumulation of IMCL has been linked to increased insulin resistance [60], the significance of EMCL remains unclear [21]. Insulin resistance can lead to diabetes onset as well as cardiovascular disease, with a 12-fold increase of diabetes onset when weight is carried from childhood to adulthood [6]. Though IMCL (and possibly EMCL) may be risk factors of metabolic disease, their metabolism in obesity is unclear [44, 45]. Present studies examining IMCL and EMCL use during exercise did not account for the fed state of participants or control for diet macronutrient composition, factors which both affect IMCL and EMCL use. Knowing how exercise affects IMCL and EMCL use in obesity could be important in understanding underlying differences in disease risk between those with childhood and adulthood onset obesity. Therefore, the aim of this preliminary study is to

understand how acute exercise affects IMCL and EMCL use in those with either childhood- or adulthood-onset obesity.

3. Objective

To conduct a pilot study that examines if males with childhood- vs. adulthood-onset of obesity has different rates of IMCL and EMCL usage in response to exercise.

4. Hypothesis

We hypothesize that:

1) Compared to those with obesity onset in adulthood, those with childhood onset obesity will have a smaller decrease in IMCL content in response to a bout of fasted exercise.

2) There will be no changes in EMCL in both groups post exercise.

5. Methods

5.1 Participants

A total of 10 male participants were recruited for this study; five participants with childhood-onset obesity (n=5, childhood onset) and five participants with adulthood-onset obesity (n=5, adulthood onset). Age of obesity onset was confirmed with the participants' medical records [3], pictures, or the Collins childhood body rating scale [61]. Childhood onset obesity was defined as having developed obesity during the pre- or peri-puberty period (9-14y) [62, 63], whereas adulthood onset obesity was defined as having been overweight after the age of 18y [62]. Participants were all physically inactive, classified as having sedentary lifestyles (>1.0-1.5 METs) [64, 65] , ≥23 years old, and had a body mass index (BMI) ≥30kg/m^2. Participants were excluded if they smoked, took any medications affecting metabolism or had underlying conditions that affected energy metabolism (e.g. diabetes, hyperthyroidism, and hypothyroidism). The study was

approved by the University of Concordia Human Research Ethics Committee, and all participants provided written informed consent.

5.2 Study Visit

All study visits were conducted at the PERFORM Centre of Concordia University (Figure 1). After an overnight fast, indirect calorimetry and total body composition using dual-energy x-ray absorptiometry were conducted. Participants were then provided with meals for three days prior to returning for the MRS study visit. On day 4, participants arrived fasted for the MRS study visit. Participants underwent a baseline measurement of IMCL and EMCL in the vastus lateralis via 1H-MRS. Participants then cycled at moderate intensity (40-59% heart rate reserve [50]) for 90 minutes. Energy expenditure was assessed during exercise via indirect calorimetry. The MRS scan of the vastus lateralis was repeated immediately post exercise to measure IMCL and EMCL levels.

5.3 Body Composition

After an overnight fast, height (cm) and weight (kg) were taken from the participant. Total and regional body composition was assessed via dual-energy x-ray absorptiometry (GE Healthcare; Lunar Prodigy Advance, Madison, Wisconsin) with Encore software (version 14.10; GE Healthcare). The leg was the region of interest we were particularly interested which is measured from the greater trochanter to the lateral malleolus [66]. Calibration was performed using manufacturer-supplied phantoms. Participants wore light clothing and removed any metallic objects. The participant then lay supine on the scanning bed with their arms placed apart from the body. If participants did not fit within the indicated scanning areas they were positioned with their left arm (at the collar bone) outside the indicated scanning region [67]. The DXA then used the scanned half of the body to produce the assessment of the other side [67].

5.4 Diet Intervention

Since the macronutrient composition of diets affects substrate oxidation [68], participants were provided with 3 days of meals prior to the MRS measurements. By controlling for 3 days, we removed the possibility of over or under consumption of nutrients which could alter substrate oxidation [69]. The diet was composed of 30% fat, 50% carbohydrate, and 20% protein, keeping with standard North American dietary patterns [70, 71]. Participants' energy expenditure was calculated by a registered dietitian using the Mifflin equation [72, 73], which is recommended for obesity [74]. Any allergies, intolerances or dietary preferences were taken into account when preparing diets. Participants were instructed to eat nothing outside of the given food and snacks.

5.5 Substrate Oxidation via Indirect Calorimetry at rest and during exercise

Energy expenditure and substrate oxidation were assessed via indirect calorimetry at rest [75] and during exercise [44]. Energy expenditure and substrate oxidation measurement at rest was assessed using a FMS with an FK-500 flow kit (Sable Systems International, Las Vegas, NV). Calibration of the indirect calorimeters was conducted prior to each measurement. For the indirect calorimetry measurement at rest, participants arrived after an overnight fast, they were asked to rest for 60 minutes prior to assessment. Once the test began, a ventilated hood was placed over the participants head for the 35 minutes measurement. The participant was asked to stay awake during the procedure and avoid movement. The first 10 minutes of measurement was removed during data analysis for acclimatization [69]. Overall, 25 minutes of indirect calorimetry was used for data analysis.

While cycling, exercise substrate oxidation was measured over 3 timepoints of 10 minutes (20^{th}, 50^{th} & 80^{th} minute) using Medgraphics Cardio O2 Metabolic Cart (MGC Diagnostics , Saint-Paul, Minnesota), Mortara ECG (Mortara Instrument Inc, Milwaukee, Wisconsin) to

measure gas exchange. The indirect calorimeter was calibrated using a 3L syringe for the flowmeters zero calibration, a calibration gas was used for span and delay alignment [75]. While the participant cycled, a mask connected to the metabolic carts was placed on the participant covering their nose and mouth [44]. Participants were asked to breathe normally and continue with cycling.

The following formulas were used to calculate substrate oxidation [76]:

Rate of glucose oxidation (g/min) = 4.59 VCO_2 (l/min) – 3.23 VO_2 (l/min)

Rate of fat oxidation (g/min) = -1.70 VCO_2 (l/min) + 1.70 VO_2 (l/min)

5.6 Exercise Protocol

Participants cycled while fasted on a recumbent bike (TechnoGym, Fairfield, New Jersey) for 90 minutes at moderate intensity [44, 49] (40-59% heart rate reserve [50]). A recumbent bike was used instead of an upright bike for comfort for our participants [77]. Prior to biking, resting heart rate was taken in order to measure target heart rate for exercise. The following equations from the American College of Sports Medicine guidelines (11^{th} edition) were used [50]:

Target Heart rate= [(Heart rate max- Heart rate rest) x %intensity] + Heart Rate rest

Heart rate max= 207 – (0.7 x age)

Participants were allowed to drink water while biking. Participant were supervised throughout the exercise to verify that they were maintaining target heart rates.

5.7 Magnetic Resonance Spectroscopy (MRS)

Measurements were taken with magnetic resonance spectroscopy (MRS) (GE Discovery MR750 3.0T) for IMCL and EMCL quantification, as previously described [37]. MRS is based on the chemical shift between fat and water within the muscle [57]. By looking at the relative

amplitude of lipid and water peaks in the spectrum, lipid levels can be assessed non-invasively [57]. MRS detects the carbon-13 found within fat molecules [78], although the abundance of carbon-13 is only ~1.1% and has low sensitivity [79]. Carbon-13 is found in most organic molecules in the body and are able to resonate in the magnetic field due to hyperpolarization [78]. The hydrogen protons found within a triglyceride molecule also contribute to MRS signal. At different voxel positioning, these protons can have different magnetic fields, therefore, it is vital to place the voxel at the same location when measuring pre and post [57]. Briefly, the participant was positioned on the table with their leg placed in the MRS machine with a coil to amplify the signal. The area of the vastus lateralis corresponded to one third the distance between the patella and ileum crest [37]. We marked the location on the muscle to ensure replicable measurements, with repeated measurement, different placement of a few degrees can change the content of the voxel [80]. A fast-gradient-echo-based localizer guided a 20x20x20cm/mm region of interest in the right vastus lateralis, taking 30 slices per plane with 5mm slice thickness. Three voxel measurements were taken and the mean was used for analysis. This measurement was repeated twice, pre and post-exercise. MRS data was processed using MATLAB (The MathWorks Inc, Natick, Massachusetts) and jMRUI [81].

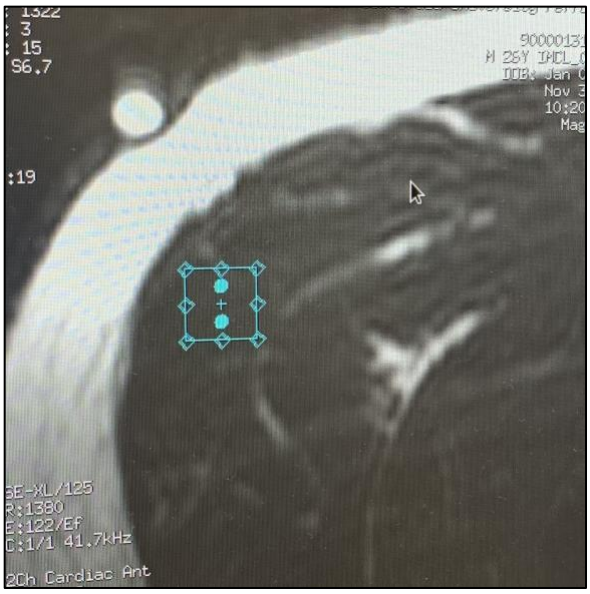

Figure 1. MRS voxel placement. Voxel indicated by blue square 20x20x20cm/mm. Small marker seen just outside the leg.

5.8 Statistical analysis

Data was tested for normality using the Shaprio-Wilks test. Unpaired t-test was used to examine the differences in childhood vs. adulthood onset. Paired t-test was used to compare changes within IMCL & EMCL post exercise per group as well as combined group. Pearson correlation coefficients were used to assess relationships between lipid oxidation and IMCL and EMCL, as well as IMCL, EMCL and fat mass and leg fat mass. Statistical significance was defined as $p<0.05$ and a confidence interval of 95% was used. All data is expressed as mean ± standard deviation. Statistical analyses were conducted using IBM SPSS Statistics 28.0.0.0 (IBM Corp, Armonk, NY, USA).

6. Results

6.1 Characteristics of participants:

Participant characteristics are shown in Table 3. A total of 10 male participants were recruited, 5 in the childhood onset and 5 in the adulthood onset group. There were no differences in age, BMI or body composition between the two groups.

Table 3. Participant characteristics.

Characteristics	Childhood-Onset (n=5)	Adulthood-Onset (n=5)	P-value
Age (y)	34.0±7.5	30.4±10.5	0.275
Weight (kg)	117.8±10.8	103.8±15.3	0.064
BMI (kg/m^2)	36.1±3.3	33.6±4.1	0.160
Total FM (kg)	37.8±11.4	36.2±4.9	0.391
Total FM (%)	33.4±7.4	36.4±3.1	0.219
Total FFM (kg)	76.9±3.3	66.6±12.1	0.066
Total leg FM (%)	25.2±3.7	29.4±4.0	0.063

Values are Mean ± SD. P-values for comparison between Childhood and Adulthood groups (independent t-test).

6.2 Resting energy expenditure and substrate oxidation:

At rest, there was no significant difference in REE (P=0.303) or RER (P=0.424) between groups (Table 4). However, lower fat oxidation in the adulthood vs childhood onset group at rest was observed but was not significant (P=0.100). There were no differences (P=0.157) in carbohydrate oxidation between groups, despite the lower measurements in the adulthood-onset group.

Table 4. Resting energy expenditure and substrate oxidation at rest.

	Childhood (n=5)	Adulthood (n=5)	P-value
REE, kcal/day	2916±272	2998±205	0.303
RER	0.82±0.1	0.83±0.1	0.424
CHOox, mg/min	200±57	169±30	0.157
FATox, mg/min	112±33	88±19	0.100

Values are Mean ± SD. P-values for comparison between Childhood and Adulthood groups. (independent t-test)

6.3 Substrate oxidation during exercise

During exercise, there were no differences (P=0.349) in RER between groups (Table 5). No differences in carbohydrate or fat oxidation were observed between groups (P=0.432, P=0.445, respectively).

Table 5. Substrate oxidation during exercise.

	Childhood (n=5)	Adulthood (n=5)	P-value
RER	0.86±0.1	0.87±0.1	0.349
CHOox, mg/min	876±417.3	846±225	0.432
FATox, mg/min	505±374	463±369	0.445

Values are Mean ± SD. P-values for comparison between Childhood and Adulthood groups. (Independent t-test)

6.4 Intramyocellular lipids and extramyocellular lipids

In the group as a whole, baseline IMCL was 4.80±1.4 A.U. and significantly (P=0.039) decreased to 4.28±1.5 A.U. post exercise (Table 6). There were no changes (P=0.352) in EMCL with exercise.

Table 6. Combined group IMCL & EMCL**.**

	Pre-IMCL	Post-IMCL	P-value	Pre-EMCL	Post-EMCL	P-value
Combined Groups	4.80±1.4	4.28±1.5	0.039*	3.02±1.4	3.12±1.4	0.352

Values are Mean ± SD. P-value showing significance within pre and post exercise levels (*p<0.05). (Paired t-test)

Figure 2 shows IMCL and EMCL values in each group pre- and post-exercise. The adulthood onset group had a baseline IMCL measurement of 4.77±1.81 A.U. which significantly decreased post exercise to 3.88±1.24 A.U. (P=0.032). In contrast, no changes (P=0.339) were observed in IMCL levels in the childhood onset group (4.83±1.27 vs 4.67±1.93 A.U., pre- vs post- exercise, respectively). With an acute bout of exercise, EMCL in both groups remained unchanged. There were no differences in IMCL or EMCL levels pre- or post- exercise between groups.

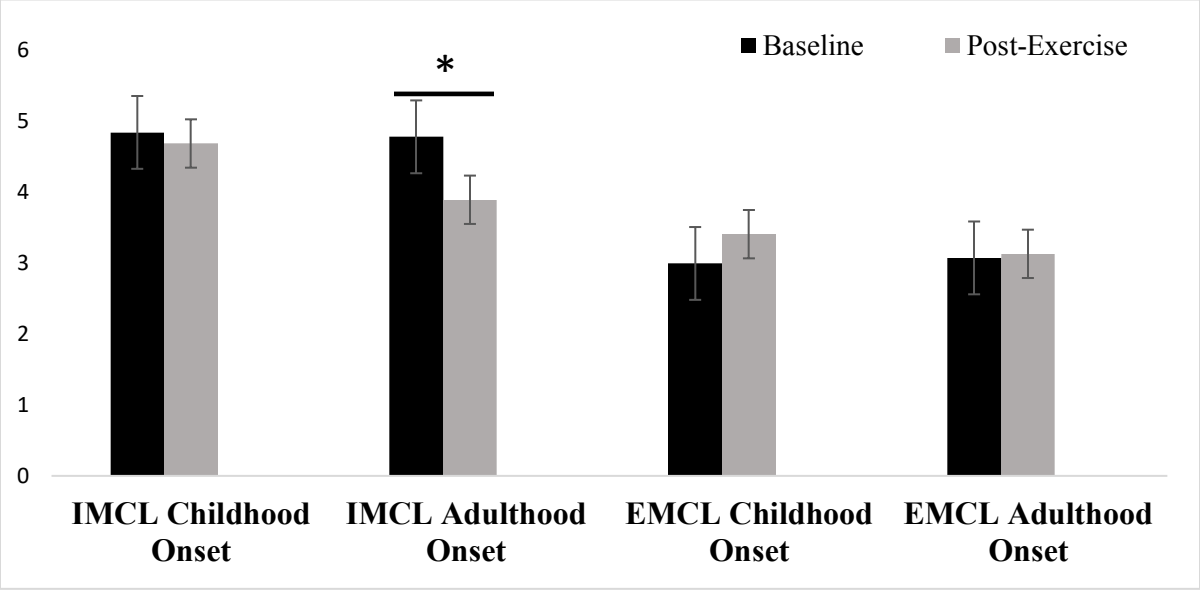

Figure 2. IMCL & EMCL changes within groups.

Comparing pre- and post- levels of IMCL and EMCL during exercise per group.

Values are Mean ± SD. P-value showing significance within group pre and post exercise levels (*p>0.05). (Paired-t test).

6.5 Relationships

The relationships between lipid oxidation at rest and during exercise and baseline IMCL and EMCL in the whole group were not significant. The relationship between baseline IMCL and EMCL and total fat and leg fat mass were not significant.

Table 7. IMCL & EMCL relationship to fat oxidation at rest, during exercise, total fat mass and leg fat mass

	Fat oxidation at rest	Fat oxidation during exercise	Total fat mass	Total leg fat mass
IMCL	0.19	0.14	0.30	0.86
EMCL	0.13	0.49	0.25	0.10

R-value providing strength of relationship Pearson correlation. No significant relationships were observed.

7. Discussion

To our knowledge, this is the first study to have compared the effects of an acute bout of exercise on IMCL and EMCL in childhood vs adulthood onset obesity. We observed, for the first time, a decreased in IMCL in adulthood onset obesity in response to exercise which was not observed in those with childhood onset obesity. This observation suggests that the age of obesity onset potentially affects IMCL use.

7.1 Energy expenditure and Resting Substrate Oxidation

In this study, we saw a trend where those with adulthood onset obesity had lower fat oxidation at rest compared to those with childhood onset obesity. We previously observed lower rates of resting fat oxidation in those with childhood onset obesity [82]. However, in this previous instance diet prior to assessment was not controlled for and could have affected substrate oxidation [82]. Ingestion of a high fat diet has been shown to increase fat oxidation, whereas, ingestion of carbohydrates decreases fat oxidation in the days prior to measurement [42]. Lipid oxidation has also been shown to decrease with obesity [10, 83, 84]. The inability to properly oxidize lipids can contribute to increased IMCL, insulin sensitivity, and cardiovascular disease risk [9, 18-20].

Exercise intensity was set at 40-59% heart rate reserve [50] to favor lipid oxidation. During moderate intensity exercise, lipolysis increases threefold [85]. We saw no differences in substrate oxidation during exercise between both groups. Though, it has been shown that consistent exercise training increases lipid oxidation at rest and during exercise in lean subjects [83]. However, little is known in individuals with obesity. Since the oxidation of IMCL has been shown to decrease with the onset of obesity [9], future studies may want to examine how exercise could improve lipid oxidation in individuals with obesity.

7.2 *IMCL & EMCL*

Interestingly, despite no differences in baseline IMCL or lipid oxidation during exercise between those with childhood and adulthood onset obesity, exercise resulted in significant IMCL decreases in individuals with adulthood onset obesity only. This finding indicates potential differences in sources of lipid used during exercise in these two groups with those with

adulthood onset obesity being more effective at utilizing IMCL as a source of energy. There are very few studies that examined IMCL use in obesity in response to exercise and none that have examined the effects of age of obesity onset. Previously, the two studies found that examined changes in IMCL in response to acute exercise in those with obesity have found none [44, 45]. Factors such as exercise duration, intensity, and fasting could affect IMCL use and account for the variations in observations between this and other studies. We controlled for macronutrient intake during the 3 days prior to assessment and our participants exercised while fasted. Additionally, we examined IMCL within the vastus lateralis, whereas Ipavec-Levasseur et al., [44] used the soleus muscle. Although the soleus does contain a high proportion of IMCL, in both upright cycling and recumbent bike cycling, the vastus lateralis is the major muscle worked [44, 86]. Another critical factor contributing to IMCL use is exercise duration. Both Levasseur et al., [44] and Gan et al., [45] saw no changes in IMCL with 60 and 40 minute bouts of exercise in individuals with obesity. Our study participants were supervised throughout the 90 minutes of cycling. The shorter duration of exercise in previous studies could have limited the ability for IMCL to be used as a fuel source. A specific time and intensity where IMCL is optimally used during exercise remains unclear [87]. Our results indicate that IMCL may be a more significant source of energy in longer duration of exercise.

In line with our hypothesis and other studies, there were no changes in EMCL with exercise in either group. Haus et al., [24] saw no changes in EMCL post 7 days of aerobic exercise in those living with obesity. Within lean populations, a 90 minute treadmill run at 60% VO2max similarly found no effect on EMCL [88]. To our knowledge, no studies have examined the effects of an acute exercise bout on EMCL use in those living with obesity. We suggest that since EMCL is stored outside the muscle fibre and further away from the mitochondria, the

location of EMCL relative to the mitochondria might result in EMCL being an ineffective source of lipid for energy [15]. The significance of EMCL remains to be elucidated. Park et al., [15] found a relationship between arterial stiffness and an accumulation of EMCL in men who were overweight and living with obesity. The association between EMCL accumulation and the risk of arterial stiffness was also seen in lean individuals [89]. Arterial stiffness is a marker for diabetes, cardiovascular disease, stroke, and mortality [90]. Presently, the results of our study and the literature indicate that EMCL may be more of an energy storage or endocrine depot.

7. 3 Strengths and Limitations

7.4 Strengths

A major strength of our study was the use of MRS to quantify IMCL and EMCL. MRS is a non-invasive and accurate method of obtaining data on IMCL and EMCL simultenaously. Additionally, the voxels were carefully placed on the vastus lateralis, ensuring that during both MRS sessions the same location was assessed. Our groups were matched for both age and body composition, which helped to eliminate confounding variables. In addition, controlling for the experiemental diet prior to exercise, ensured that macronutrient oxidation would be consistent, and thus eliminating errors introduced by consuming different diets. Lastly our exercise session was supervised and done while participants were fasted for optimal fat oxidation.

7.5 Limitations

As this was a pilot study, we only had 5 participants per group which could have affected the ability to observe differences and relationships between variables. However, we were sufficiently powered to see a decrease in IMCL in those with adulthood onset obesity. Also, the present study only included males since males and females tend to have different rates of

substrate oxidation [41, 91]. Circulating estrogen in females could be a factor in substrate utilization and selection during exercise [92] as different phases of the menstrual cycle have higher estrogen levels, which may promote lipolysis [93]. More importantly, females have been found to have lower RER values during exercise, relying more on lipids as a fuel source [41, 94, 95]. Though only including males in this pilot study minimized the variation that would have been introduced in including both sexes, the study should be repeated in females. Repeating the study in females would provide a larger picture of how age of obesity onset affects IMCL and EMCL utilization in individuals with obesity.

8. Conclusion

In this pilot study, we made the novel observation that males with childhood onset obesity do not use their IMCL stores the same way as those with adulthood onset obesity. This finding is especially significant given that our groups were similar in age and body composition and that the experimental diet and exercise were controlled for. Clinically, our results suggest that there may be potential perturbations in IMCL metabolism in those with childhood onset obesity as the use of IMCL with exercise in those with adulthood onset obesity were more reflective of that in lean participants. These observations suggest that treatment of obesity for those with childhood onset should be different to those with adulthood onset obesity. These results serve as a launching point for future studies to examine IMCL and EMCL metabolism in obesity.

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