Applied Physiology, Nutrition, and Metabolism

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Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2018-0635.R2
Manuscript Type:	Review
Date Submitted by the Author:	30-Nov-2018
Complete List of Authors:	Beaudry, Kayleigh; University of Waterloo Faculty of Applied Health Sciences, Kinesiology Devries, Michaela; University of Waterloo Faculty of Applied Health Sciences, Kinesiology
Keyword:	sex differences, IMCL, intrahepatic triglyceride (IHTG), fat oxidation, exercise, NAFLD, type II diabetes, metabolism
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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Manuscripts

Sex-based differences in hepatic and skeletal muscle triglyceride storage and metabolism

Kayleigh M Beaudry, Michaela C Devries

Department of Kinesiology, Faculty of Applied Health Sciences, University of Waterloo,
Waterloo, ON, Canada

Corresponding author:

Michaela C Devries
Department of Kinesiology, BC Matthews Hall
University of Waterloo
200 University Ave W
Waterloo, ON
N2L 3G1
m4devrie@uwaterloo.ca
Phone: 519-888-4567 x 37925

Additional author information:

Kayleigh Beaudry
Km2beaudry@uwaterloo.ca

Draft

Abstract:

Women and men store lipid differently within the body with men storing more fat in the android region and women storing more fat in the gynoid region. Fat is predominately stored in adipose tissue as triacylglycerides (TG); however, TG are also stored in other tissues including the liver and skeletal muscle. Excess hepatic TG storage, defined as a TG concentration $> 5\%$ of liver weight and known as non-alcoholic fatty liver disease (NAFLD), is related to the metabolic syndrome. Similarly, elevated skeletal muscle TG, termed intramyocellular lipids (IMCL), are related to insulin resistance in obesity and type II diabetes. Men store more hepatic TG than women and, unsurprisingly, NAFLD is more prevalent in men than women. Women store more IMCL than men, yet type II diabetes risk is not greater, which is likely due to the manner in which women store TG within muscle. Sex-based differences in TG storage between men and women are underpinned by differences in mRNA expression, protein content and enzyme activities of skeletal muscle and hepatic lipid metabolic pathways. Furthermore, women have a greater reliance on lipid during exercise due to upregulation of lipid oxidative pathways. The purpose of this review is to discuss the role of sex in mediating lipid storage and metabolism within skeletal muscle and the liver at rest and during exercise and its relationship with metabolic disease.

Keywords: sex differences, IMCL, intrahepatic triglyceride (IHTG), fat oxidation, exercise, NAFLD, type II diabetes, metabolism

Introduction:

Sex-based differences in adipose storage are well recognized with men storing more adipose in the android region and women storing more adipose in the gynoid region of the body (Karastergiou et al. 2012). While fat is predominately stored within adipose tissue, it is also stored ectopically in other tissues including the liver and skeletal muscle. Based on the differences in adipose tissue distribution it is not surprising that ectopic lipid storage is greater in muscle in women (Devries et al. 2007; Tarnopolsky et al. 2007) and appears to be greater in the liver in men (Pan and Fallon 2014; Mittendorfer et al. 2016). Sex-differences in ectopic lipid storage are related to differences in hepatic and muscle metabolism and influence substrate utilization at rest and during exercise. Furthermore, elevated ectopic lipid storage is associated with metabolic disease and thus differences in storage may be related to sex-based differences in disease development and progression (Table 1). In this review we discuss the role of sex in mediating ectopic lipid storage in relation to resting and exercise metabolism as well as how these differences influence the risk of metabolic disease in both sexes.

Skeletal Muscle Lipid Storage

Lipids are stored in muscle as intramyocellular lipids

Fat is stored in skeletal muscle (Schrauwen-Hinderling et al. 2006) within lipid droplets (LD) termed intramyocellular lipids (IMCL) (Schrauwen-Hinderling et al. 2006)]. IMCL are an important intracellular source of energy to working muscles, analogous to muscle glycogen (Hoppeler 1986; Schrauwen-Hinderling et al. 2006). IMCL content increases with endurance training (ET) and is higher in athletes as compared with sedentary individuals (van Loon and Goodpaster 2006), providing further support that IMCL are an important fuel source to support muscle metabolic needs. However, increased fatty acid (FA) intake also increases IMCL content,

suggesting that skeletal muscle may also represent a storage depot for fat during periods of fat oversupply (Schrauwen-Hinderling et al. 2006). In obese individuals, higher IMCL content is associated with insulin resistance (IR); however, this relationship is in contrast to endurance athletes, who have high insulin sensitivity [IS; (van Loon 2004)]. This “athletes’ paradox”, where highly IS, endurance athletes have a similar IMCL content as IR, obese and type 2 diabetic (T2D) individuals (Dube et al. 2008; Coen and Goodpaster 2012; Daemen et al. 2018) is an active area of research with recent evidence suggesting that it is not IMCL content *per se* that is related to IR, but the localization of the droplets within the myocyte (Bergman et al. 2012; Samjoo et al. 2013; Daemen et al. 2018).

Skeletal muscle lipid metabolism

Fatty acids, derived primarily from adipocyte TG lipolysis and dietary intake, are taken up into skeletal muscle by passive diffusion and by FA transporters: FAT/CD36, FABPpm and FA transport proteins (FATP1 and FATP4) (Watt and Hoy 2012). Once in the myocyte, FA is converted to FA-CoA by acyl-CoA synthetase and can either be incorporated into IMCL for storage or can enter the mitochondria for oxidation (Bosma et al. 2012; Coen and Goodpaster 2012). In order to undergo oxidation, FA are transported into the mitochondria by carnitine palmitoyl transferase I (CPTI), translocase and CPT2 (Goodpaster et al. 2001; Coen and Goodpaster 2012). When FA are used to form TG, three FA are added to a glycerol backbone, a process regulated by glycerol-3-phosphate acyl transferase [GPAT, (Sul and Wang 1998)]. Perilipin (PLIN)2 is also important in TG synthesis, promoting TG synthesis and inhibiting lipolysis (MacPherson and Peters 2015). Alternatively, IMCL breakdown is controlled predominately by adipose triglyceride lipase (ATGL), with hormone sensitive lipase (HSL) and monoacylglycerol lipase (MAGL) also contributing (Zechner et al. 2012; Shaw et al. 2013).

PLIN3 and 5 also help regulate TG breakdown by mediating IMCL-mitochondria apposition (PLIN5) and controlling the delivery of FA to the mitochondria (PLIN3), which helps couple TG breakdown to oxidation, preventing the accumulation of lipid byproducts (MacPherson and Peters 2015).

Skeletal muscle lipid storage location differs based on training and disease state

IMCL are stored within skeletal muscle between myofibrils (intermyofibrillar – IMF) or below the sarcolemma (subsarcolemmal – SS). Studies using electron microscopy have found that IMCL stored in the SS region are related to IR, particularly among obese adults (Nielsen et al. 2010; Samjoo et al. 2013; Chee et al. 2016). Furthermore, patients with T2D store more IMCL in fewer but larger LD located primarily in the SS region of type II, not type I fibres (Daemen et al. 2018). Indeed, SS IMCL content is 3x greater in T2D as compared with non-diabetic, BMI matched and elite endurance-trained controls with no difference in IMF IMCL content between groups (Nielsen et al. 2010). Morphologically, lipids in the IMF are often found associated with mitochondria whereas lipids in the SS are often found touching the sarcolemma, suggesting that IMF IMCL may be primed for oxidation whereas SS IMCL may interfere with insulin signaling (Nielsen et al. 2010). Indeed, athletes tend to store a larger number of small LD in type I fibers, primarily located in the IMF (Daemen et al. 2018). ET reduces SS IMCL content (Nielsen et al. 2010; Devries et al. 2013; Samjoo et al. 2013), redistributing it to the IMF and improves IS in T2D and obese men (Nielsen et al. 2010; Samjoo et al. 2013), but not obese women (Devries et al. 2013). However, studies using fluorescence microscopy have shown that centrally located IMCL are inversely related to glucose disposal rate following a period of overfeeding (Covington et al. 2017). These studies have also found that IMCL storage increases from the centre of the myofibril towards the sarcolemma and that elite athletes have a greater

amount of IMCL stored in the peripheral area of the myocyte as compared with sedentary or T2D individuals (van Loon 2004). These findings are in direct contrast to the findings from electron microscopy studies and are limited by the fact that this method does not allow direct visualization of the SS and thus may not have had the sensitivity necessary to distinguish between SS and peripheral IMF, the area just internal to the SS region, IMCL. Indeed, Nielsen et al. (2010) found that mitochondrial volume density is greatest in the peripheral IMF, suggesting that since IMF IMCL are in close proximity with mitochondria, that IMCL content may also be higher in this area. More work is needed carefully delineating SS, peripheral IMF and central IMF regions to elucidate the role IMCL storage location plays in relation to IR.

Lipid droplet size and its relation to disease

IMCL size is also related to IR, with larger IMCL droplets being negatively correlated with IS (Chee et al. 2016; Covington et al. 2017; Daemen et al. 2018). Specifically, Chee et al. (2016), found that the 3-fold greater IMCL content in the SS region in older obese individuals compared to young and old lean individuals was due to an ~25% greater IMCL size, which was closely related to IR. Greater IMCL size could be related to a reduced capacity to oxidize lipid due to a lower LD surface area to volume ratio (van Loon 2004)]. It is also speculated that large LD could create structural barriers that obstruct GLUT4 translocation and docking/fusing with the sarcolemma, decreasing skeletal muscle glucose uptake (van Loon 2004; van Loon and Goodpaster 2006). The relationship between IMCL size and IR is further supported by research showing that IMCL size decreases and IS increases in response to ET in T2D (Daemen et al. 2018) and weight loss (He et al. 2004).

Sex differences in IMCL storage

Women have a greater IMCL content than men (Roepstorff et al. 2002; Steffensen et al. 2002; Devries et al. 2007; Tarnopolsky et al. 2007). Given this higher IMCL content, it could be speculated that women may be at greater risk of developing T2D than men; however, studies have shown that the risk of IR and T2D is lower in women, despite greater total body adiposity (Chen et al. 2012; Kautzky-Willer et al. 2012; Sattar 2013; Kautzky-Willer et al. 2016). Furthermore, elevated IMCL in women does not impair whole body or leg IS (Høeg et al. 2009). The lower risk of T2D in women may be mediated by estrogen as risk for T2D increases in women post-menopause when estrogen concentrations decline (Muka et al. 2017). While numerous factors likely underpin this sex-based difference in disease risk, differences in how men and women store and oxidize lipid in muscle, which is influenced by estrogen (Devries et al. 2005), may also influence disease risk. Indeed, electron microscopy studies have shown that the higher IMCL content in women is due to a greater number of IMCL, not a greater IMCL size (Devries et al. 2007; Tarnopolsky et al. 2007). As detailed above, greater IMCL size is related to IR (Chee et al. 2016; Covington et al. 2017), thus since IMCL size is not different between men and women, it is not surprising that women are not at a greater risk of developing T2D.

Sex differences in muscle fibre type

Sex differences in muscle fibre type composition may be another reason why T2D risk is not greater in women despite greater IMCL content. Women have a greater amount of type I muscle fibres than men (Steffensen et al. 2002; Lundsgaard and Kiens 2014). Type I fibres are more oxidative than type II fibres due to increased oxidative enzyme activity and have a greater capacity for lipid uptake, storage and oxidation (Malenfant et al. 2001). There is a reported 2-3x greater lipid content in type I versus type II fibers, and yet type I fibers are more IS (van Loon 2004). The greater IMCL content in type I fibres could in part explain the greater IMCL content

and IS in women as it has been reported that women have a larger type I fiber area compared to men, which is associated with higher IMCL content (Steffensen et al. 2002). Although the effect of fibre type distribution and its effect on IMCL has been disputed (van Loon and Goodpaster 2006; Tarnopolsky et al. 2007), a fibre type distribution difference between men and women may contribute in part to the effect of sex on IMCL storage and IR and warrants further examination.

Sex differences in skeletal muscle lipid metabolism

Sex comparative studies have shown that metabolic pathways related to muscle FA uptake, IMCL synthesis and degradation and FA transport into mitochondria and oxidation are upregulated in women (Figure 1). Women have greater mRNA expression and protein content of FABP, FATP and FAT/CD36 as compared with men (Luxon and Weisiger 1993; Fu et al. 2009; Lundsgaard and Kiens 2014). The localization of FAT/CD36 is also important to consider as FAT/CD36 is highly correlated with IMCL storage in human skeletal muscle (Lundsgaard and Kiens 2014). In T2D and obese individuals FAT/CD36 relocates to the sarcolemma, which contributes to an increased rate of FA transport into skeletal muscle and results in an excess accumulation of IMCL (Bonen et al. 2004). Whether FAT/CD36 sarcolemmal content differs between the sexes has not been examined.

Women have higher mRNA content of SREBP-1 and mtGPAT (Fu et al. 2009) and PLIN2 protein content (Peters et al. 2012), suggestive of a greater capacity for IMCL synthesis. IMCL lipolytic capacity is also greater in women as evidenced by a greater ATGL activity (Moro et al. 2009) and HSL mRNA expression and protein content, but not HSL activity (Roepstorff et al. 2006). Furthermore, PLIN3 and PLIN5 protein content are also higher in women, suggesting that women have a greater ability to link lipid breakdown with oxidation, preventing lipid byproduct accumulation (Peters et al. 2012). A greater capacity to oxidize fat

within skeletal muscle is also supported by sex differences in other key players of fat oxidation including CPT-1, trifunctional protein (TFP-1 α) and very long-chain acyl-CoA dehydrogenase (VLCAD) (Fu et al. 2009; Maher et al. 2009; Maher et al. 2010). Together these findings provide a metabolic basis by which women have a greater capacity to store and oxidize fat within skeletal muscle and suggest that women have a greater IMCL flux than men.

Hepatic Lipid Storage and its relationship with non-alcoholic fatty liver disease

Hepatic Lipid Storage

Fat is stored within the liver in LD termed intrahepatic triglycerides (IHTG). Normal physiological IHTG content is defined histologically as macroscopic steatosis present in < 5% of hepatocytes, and with ¹H-MRS and MRI as liver fat < 5.56% and < 6–6.4%, respectively (Petäjä and Yki-Järvinen 2016). Imbalances in hepatic lipid metabolism resulting from increased TG synthesis or decreased lipolysis and oxidation lead to pathological accumulation of IHTG (Gluchowski et al. 2017), known as non-alcoholic fatty liver disease (NAFLD). The prevalence of NAFLD is 20-30% in the general population; however, this increases to 80-90% in obese individuals (Bellentani et al. 2010). NAFLD is closely associated with many features of the metabolic syndrome, including IR (Giorgio et al. 2005; Chen et al. 2006). While NAFLD can follow a benign course, some individuals progress to non-alcoholic steatohepatitis, liver fibrosis and cirrhosis (Pan and Fallon 2014). Furthermore, risk of cardiovascular and liver-related death is greater in those with NAFLD, particularly those with concomitant metabolic syndrome (Pan and Fallon 2014).

Hepatic TG metabolism

Lipid stored within the liver originates from dietary fat, *de novo* lipogenesis (DNL) or FA released from adipocytes (Mashek 2013). In the fed state, the formation of FA via DNL is stimulated by insulin and mediated by acetyl CoA carboxylase and FA synthase. FA in the liver can be used to synthesize TG, mediated by diacylglycerol O-acyltransferase (DGAT)2 (Qi et al. 2012; Wurie et al. 2012). Upon synthesis, hepatic TG is packaged into VLDL particles to be delivered to other tissues, or remains within the liver stored within LD. PLIN2 promotes TG accumulation in the liver by increasing TG synthesis, inhibiting lipolysis, and preventing the incorporation of TG into VLDL (Magnusson et al. 2006). Alternatively, in the fasted state FA within the liver can undergo β -oxidation, with the resulting acetyl CoA entering the TCA cycle or used to synthesize ketone bodies.

Alterations in hepatic lipid metabolism lead to NAFLD

NAFLD is characterized by the presence of excess, very large LD (Kochan et al. 2015; Gluchowski et al. 2017). The excess IHTG stored in NAFLD is due to increased FA availability resulting from increased adipocyte FA release and increased DNL (Donnelly et al. 2005). IR is a hallmark of NAFLD (Gastaldelli 2017; Kitade et al. 2017). In the IR state insulin fails to suppress adipocyte lipolysis, resulting in increased FA release and uptake into the liver (Gastaldelli 2017). Excess adipocyte FA release is thought to contribute to ~60% of IHTG stored in NAFLD (Donnelly et al. 2005). Interestingly, in NAFLD DNL is not suppressed despite IR, increasing the contribution of DNL to IHTG synthesis from 5% in healthy individuals to 25% in those with NAFLD (Donnelly et al. 2005; Fabbrini et al. 2010). Increased FA availability within the liver in NAFLD is accompanied by increased PLIN2 content (Straub et al. 2008), which promotes TG synthesis and inhibits TG breakdown and incorporation of TG into VLDL (Magnusson et al. 2006; Imai et al. 2007), decreasing TG secretion from the liver. Lastly,

impaired FA oxidation also contributes to the development of NAFLD, resulting in part due to increased malonyl CoA production via DNL, which inhibits CPT-1 activity, reducing FA entry into the mitochondria and subsequent oxidation (Wei et al. 2008). Together, current data suggests that NAFLD results from increased TG synthesis, decreased TG breakdown and oxidation and decreased VLDL-TG secretion.

Sex Differences in Hepatic Lipid Storage

NAFLD prevalence is higher in men than women, ranging from 4.3%-42% and 1.6%-24% in men and women, respectively, depending on the defining criteria (Pan and Fallon 2014). The lower prevalence of NAFLD in women is speculated to be, at least in part, due to the protective effects of estrogen. Animal studies have shown that estrogen treatment to male or oophorectomized female rats decreases IHTG content (Toda et al. 2001; Kim et al. 2015; Jin et al. 2017). Furthermore, the risk of developing NAFLD increases in women post-menopause when estrogen decreases (Ryu et al. 2015). However, whether men inherently store more hepatic lipid than women is unclear. A recent study of 233 adults across the spectrum of obesity found that IHTG content in men was double that of women [11% vs 5%, (Mittendorfer et al. 2016)]. While not statistically significant, likely due to the wide range of adiposity of included participants, this difference existed despite a greater percentage of women being obese (women 73%, men 58%). Similarly, while Westerbacka et al. (2004) found no difference in IHTG content between men and women (8.9% vs 6.7%, respectively); the men and women were weight-matched, and thus the women were obese (BMI 31, 35% body fat), whereas the men were normal weight (BMI 26, 21% body fat). Considering that IHTG storage increases with increasing adiposity (Devries et al. 2008; Elisa et al. 2010), the findings of these studies suggest that for a given level of adiposity IHTG storage is lower in women. Indeed, a direct comparison of lean

and obese men and women showed no difference in liver fat between normal weight men and women (20% and 32% body fat, respectively) or obese men and women (34% and 49% body fat, respectively); however, liver fat was higher in obese men than lean women, despite having similar amounts of body fat (Devries et al. 2008). While these differences may be related to the fact that women naturally store more body fat than men, they may also be due to the fact that men store more android body fat, in close proximity to the liver, or underlying sex differences in hepatic fat metabolism (Marinou et al. 2011; Pramfalk et al. 2015). Further work comparing IHTG content in healthy individuals accounting for body fat is required to determine the effects of sex on IHTG content. However, underlying metabolic differences in hepatic metabolism and IHTG storage between the sexes may be, at least in part, responsible for this observed sex-based difference in disease risk.

Sex differences in hepatic lipid metabolism promote lipid storage in men and lipid oxidation in women

While the effect of sex on IHTG content is unclear, there are well-described sex-based differences in hepatic metabolism that support the hypothesis that men store more IHTG than women (Figure 2). Women have a greater rate of hepatic fat oxidation as evidenced by a greater production of 3-hydroxybutyrate in the fasted and fed state (Marinou et al. 2011; Pramfalk et al. 2015). Furthermore, while no difference in the contribution of plasma or dietary FAs to VLDL-TG has been reported between insulin sensitive men and women (Hodson et al. 2007), men have a greater contribution of DNL to VLDL-TG in the postprandial, but not fasted state (Pramfalk et al. 2015). Findings from animal studies suggest that this may be due to greater SREBP-1c expression and activity (Marinis et al. 2008; Stöppeler et al. 2013), but not greater ACC or FA synthase mRNA (Marks et al. 2013). Increased rates of DNL are hypothesized to be involved in

the development of NAFLD (Lambert et al. 2014) with a greater amount of DNL-produced TGs being incorporated into VLDLs (15%) in individuals with NAFLD as compared with healthy controls [2-5%, (Diraison et al. 1997; Diraison et al. 2003)]. Furthermore, while VLDL-apoB-100 secretion rate is ~20% lower in women, VLDL-TG secretion is 70% higher, indicating that while women secrete fewer VLDL particles, they are more TG-rich, suggesting that women have a greater capacity to clear TG from the liver (Magkos et al. 2007). Together these data imply that women have a greater ability to remove fat from the liver by both increased FA oxidation and TG secretion, which may explain their reduced risk for the development of NAFLD.

Increased hepatic FA oxidation in women in the fasted state is likely the result of greater fasting plasma non-esterified FA in women (Marinou et al. 2011), as ketone production is directly related to plasma non-esterified FA concentration (Grey et al. 1975). However, increased plasma FA concentrations cannot be the sole reason for the greater fat oxidation by women as, while sex does influence plasma FA concentration (Pramfalk et al. 2015), plasma FA concentrations are similar between men and women in the fed state, yet women maintain an increased rate of 3-hydroxybutyrate production (Pramfalk et al. 2015). Greater hepatic uptake of plasma FA has been found in female rats (Kushlan et al. 1981) and cells (Sorrentino et al. 1992), and is, at least in part, responsible for the greater FA oxidation observed in women in the fed state. Furthermore, some (Merimee and Fineberg 1973; Merimee et al. 1978), but not all (Haymond et al. 1982; Soeters et al. 2007), studies have shown that plasma glucagon, a direct stimulator of ketogenesis, is higher in women in the fasted state than men, and thus may also contribute to the increased rate of FA oxidation in women in the fasted state. However, during hyperinsulinemia, plasma glucagon levels are similar (Amiel et al. 1993) or lower (Davis et al. 1993) in women, suggesting that sex differences in glucagon are not responsible for higher levels

of fat oxidation by women in the fed state. No sex difference in plasma insulin concentrations, which inhibits ketogenesis indirectly through its inhibitory effect on adipocyte lipolysis, in the fasted or fed state have been reported (Merimee and Fineberg 1973; Magkos et al. 2007; Soeters et al. 2007).

Sex influences the effects of exercise on IMCL and IHTG content and utilization

Sex differences in skeletal muscle lipid utilization during exercise

It is well established that during exercise women rely more on lipid as fuel as compared with men as evidenced by a lower respiratory exchange ratio (Devries et al. 2007; Tarnopolsky et al. 2007)] However, the metabolic site of increased lipid reliance during exercise in women remains unknown. Some studies have found no sex-based differences in plasma FA utilization during exercise (Friedlander et al. 1999; Burguera et al. 2000; Romijn et al. 2000). However, one study by Roepstorff et al. (2002) found that during the final 60 min of a 90 min cycling bout women oxidized 47% more plasma FA compared to men. Furthermore, considering that IMCL utilization during exercise is related to starting IMCL content (White et al. 2003), it could be assumed that women, who store more IMCL than men (Tarnopolsky et al. 2007), would utilize more IMCL during exercise. However, the research is controversial, with studies showing that women utilize more (Roepstorff et al. 2002; Steffensen et al. 2002; Tarnopolsky et al. 2007), less (Zehnder et al. 2005), or equal amounts of IMCL (Kiens et al. 1993; Bergman and Brooks 1999; Bergman et al. 1999; Guo et al. 2000; Devries et al. 2007) during exercise as men.

Is the increased reliance on lipid by women during exercise a result of greater hepatic lipid oxidation?

Hepatic lipid is not a main fuel source during exercise, contributing approximately 3-5% of total exercise energy expenditure under normal dietary conditions (Helge et al. 2001; Sondergaard et al. 2011), but its contribution can increase to ~25% of total exercise energy expenditure following adaptation to a fat-rich diet (Helge et al. 2001). However, acute exercise does not decrease IHTG content in men with NAFLD (Bilet et al. 2015). Despite a relatively small contribution to exercise energy expenditure, a potential sex difference in hepatic TG oxidation during exercise may exist. Horton et al. (2002) found that that VLDL-TG uptake into skeletal muscle was greater in women than men in the fasted and fed state. These findings are supported by the finding that LPL mRNA expression is greater in women (Kiens et al. 2004); however, LPL activity does not differ between the sexes at rest (Kiens et al. 2004; Perreault et al. 2004) and increases only in men following acute exercise (Perreault et al. 2004). Furthermore, subsequent work has shown no difference in VLDL-TG oxidation between the sexes at rest or during exercise (Sondergaard et al. 2011). Together these findings do not support a role for hepatic TG to contribute much as a fuel source during acute exercise, nor that increased IHTG utilization during exercise is responsible for the greater reliance on lipid during exercise in women.

Exercise training increases IMCL, but lowers T2D risk

Exercise training can prevent the development and progression of T2D (Canadian Diabetes Association Clinical Practice Guidelines Expert et al. 2013). An acute bout of exercise increases post-exercise glucose handling up to 20-fold for 2-72 hours, depending on exercise type, intensity and duration in IS and IR individuals (Goodyear and Kahn 1998; Richter et al. 2001; Riddell and Sigal 2013). The increase in muscle glucose uptake induced by exercise is mediated by several factors, including contraction-induced GLUT 4 translocation, depletion of

muscle glycogen stores and improvements in the regulation of hepatic glucose output (Borghouts and Keizer 2000).

Interestingly, ET increases IMCL content (van Loon et al. 2004); however, the increase in IMCL content with ET is due to a significant increase in IMCL number, not IMCL size (Tarnopolsky et al. 2007). This is advantageous because an increase in IMCL number will increase LD surface area, allowing for greater interaction between the IMCL and lipolytic proteins and mitochondria (van Loon and Goodpaster 2006; Tarnopolsky et al. 2007). Furthermore, as noted above (Chee et al. 2016; Covington et al. 2017), the effect of IMCL on IR may be related to greater IMCL size, not overall IMCL content. Thus, an increase in IMCL content due to an increase in IMCL number, not an increase in IMCL size (Tarnopolsky et al. 2007), may represent a physiological adaptation to training and may, at least partly, explain the Athlete's Paradox. Importantly, ET in T2D reduces IMCL size in line with the improvement in IS (Daemen et al. 2018), again suggesting that IMCL size, not content, is related to IR.

With ET there is also a shift in the localization of IMCL within skeletal muscle. In obese and T2D patients, 10-12 weeks of ET decreased IMCL content in the SS with no changes in total myocyte IMCL content (Nielsen et al. 2010; Devries et al. 2013; Samjoo et al. 2013; Daemen et al. 2018). Furthermore, ET results in a higher IMCL-mitochondria apposition (Tarnopolsky et al. 2007; Devries et al. 2013; Samjoo et al. 2013), suggestive of a mechanism by which training increases fat oxidation. With ET IMCL use during exercise increases (Holloszy and Coyle 1984; Phillips et al. 1996; Tarnopolsky et al. 2007), along with an increase in protein content and enzyme activities of muscle fat oxidation pathways (Devries et al. 2013; Samjoo et al. 2013), suggestive of an increased rate of IMCL turnover following ET. This is important in relation to IS as a high rate of IMCL turnover paired with an increased IMCL-mitochondria co-localization

may prevent the accumulation of lipid by-products which can negatively influence insulin signaling (Moro et al. 2008). Together these findings suggest that ET-induced increases in IMCL content differ from increases in IMCL content induced by obesity, and are not related to IR, because with ET 1) increases in IMCL content are due to increased IMCL number, not size; 2) there is an increase in IMCL-mitochondria co-localization; and 3) there is a redistribution of IMCL from the SS to the IMF region of the myocyte.

Does sex influence the effect of ET on IMCL content?

Few studies have examined whether sex influences the effect of ET on IMCL content. Tarnopolsky et al. (2007) found that ET increases mitochondrial and IMCL content similarly in both sexes (Tarnopolsky et al. 2007). Furthermore, unlike with acute exercise where IMCL-mitochondria co-localization increased only in women (Devries et al. 2007), it increased similarly in both sexes following ET (Tarnopolsky et al. 2007). No study to date has examined whether the effect of ET on IMCL storage location differs between the sexes; however, ET has been found to induce a redistribution of IMCL from the SS to the IMF in both sexes (Devries et al. 2013; Samjoo et al. 2013).

Training lowers IHTG content and may be more effective in men than women

Both endurance and resistance training are effective strategies to lower IHTG content in individuals with NAFLD independent of diet and/or weight loss (Hallsworth et al. 2011; Keating et al. 2012; Cheng et al. 2017; Katsagoni et al. 2017), with no differences in effectiveness between the two modes (Bacchi et al. 2013; Katsagoni et al. 2017). Importantly, the effect of training on IHTG content in individuals with NAFLD is similar to that of an energy-restricted, low carbohydrate diet (exercise: -24.4%, diet: -23.3%), with greater effects seen when both are

combined [-47.9%, (Liu et al. 2014)]. Interestingly, while training decreases IHTG content, there is no effect of training on hepatic lipoprotein kinetics as evidenced by no change in VLDL-TG or VLDL-apoB100 secretion rate, suggesting that the effects of training to lower IHTG content are due to increased liver fat oxidation, not decreased FA delivery to, or increased TG export from, the liver (Sullivan et al. 2012).

Whether exercise intensity influences the effect of training on IHTG content is controversial. Several studies have found that training at 45-55% $\text{VO}_{2\text{peak}}$ induced similar decreases in IHTG content as training at exercise intensities between 65-80% $\text{VO}_{2\text{peak}}$ (Keating et al. 2015; Zhang et al. 2016); whereas other studies have found greater improvements in IHTG content with higher intensity exercise (Kistler et al. 2011; Balducci et al. 2015). Furthermore, two recent meta-analyses came to opposing conclusions regarding the effect of exercise intensity on IHTG content (Orci et al. 2016; Katsagoni et al. 2017), thus whether an optimal exercise intensity exists to reduce IHTG content remains unclear. Similarly, the effect of exercise dose (a function of session duration, number of sessions/week and intervention duration) on IHTG content is also controversial with one meta-analysis finding that the exercise dose did not affect the ability of training to reduce IHTG (Orci et al. 2016), whereas another found a greater effect of moderate-high volume, as compared with low-moderate volume exercise, to reduce IHTG (Katsagoni et al. 2017). Importantly, irrespective of exercise intensity and volume, both meta-analyses found that training lowers IHTG in individuals with NAFLD (Orci et al. 2016; Katsagoni et al. 2017).

Few studies have examined whether sex influences the effectiveness of training to reduce IHTG content. In a group of lean and obese men and women without NAFLD we failed to find an effect of sex on the change in IHTG content in response to 12 weeks of ET (Devries et al.

2008). These results are confounded by the fact that training did not lower IHTG content in lean or obese participants, despite obese participants having higher IHTG content than lean participants prior to training (Devries et al. 2008). However, we did find that γ -glutamyltransferase, an enzyme elevated when liver disease is present, was lowered by ET in men, but not women, suggesting that perhaps ET may be more effective to ameliorate NAFLD in men (Devries et al. 2008). Indeed, animal studies have found that the change in IHTG content in response to ET is greater in male, as compared with female, mice (Magkos 2012). Furthermore, this hypothesis is supported by data in adolescents with NAFLD where both aerobic and resistance exercise have been found to reduce IHTG content in boys, whereas only ET was effective in girls (Lee et al. 2012; Lee et al. 2013). Much work remains to examine whether there is a sex-based effect of training on IHTG content. Given the increased risk of NAFLD in men, the suggestion that training may be especially effective in men is of clinical interest.

Conclusions

Sex influences lipid storage within the liver and muscle. Of note, it is curious that greater IHTG content in men is related to a greater risk of NAFLD, whereas greater IMCL content in women is not related to an increased risk of T2D. Given the role of lipid storage in the development of metabolic disease, the fact that lipid is stored differently due to sex-based differences in hepatic and muscle metabolism, and has a differential effect on disease risk, highlights the importance of including both men and women in trials examining the effects of lifestyle or pharmacological agents on metabolic disease outcomes.

Conflict of interest: The authors have no conflicts of interest to report

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Table 1: Summary of sex differences in hepatic and skeletal muscle metabolism in relation to disease risk

Men have a greater risk of NAFLD than women	Pan & Fallon, 2014
<i>This may be due to findings that:</i>	
Men have greater de novo lipogenesis	Pramfalk et al, 2015
Women have greater hepatic fat oxidation	Marinou et al, 2011. Pramfalk et al, 2015.
Women secrete TG-rich VLDL	Magkos et al, 2007
Women store more IMCL than men, but are not at a greater risk of T2D	Chen et al, 2012. Kautzky-Willer et al, 2012. Kautzky-Willer et al, 2013.
<i>This may be due to findings that:</i>	
Women have greater IMCL number and not greater IMCL size	Devries et al, 2017. Tarnopolsky et al, 2007.
Women have greater IMCL flux	Moro et al, 2009. Roepstorff et al, 2006. Fu et al, 2009.
IMCL breakdown is closely linked to oxidation due to greater PLIN 3 and PLIN 5 content	MacPherson & Peters, 2015. Peters et al, 2012.

Figure Legends

Figure 1: Sex differences in skeletal muscle lipid metabolism. Bolded text denotes metabolic pathways that are elevated in women as compared with men. β -ox – β -oxidation; ATGL – adipose triglyceride lipase; CPT-1 – carnitine palmitoyl transferase 1; CPT-2 – carnitine palmitoyl transferase 2; Cyt – cytosol; ETC – electron transport chain; FABP – fatty acid binding protein; FAcarn – fatty acyl – carnitine; FA-CoA – fatty acyl CoA; FAT/CD36 – fatty acid transporter/CD36; FATP – fatty acid transport protein; HSL – hormone sensitive lipase; IMCL – intramyocellular lipid; mtGPAT – mitochondrial glycerol-3-phosphate acyltransferase; PLIN5 – perilipin 5; SREBP-1c – sterol regulatory element-binding protein 1c; TAGH – triacylglycerol hydrolase; TFP – trifunctional protein; VLCAD – very long chain acyl CoA dehydrogenase.

Figure 2: Sex differences in hepatic lipid metabolism. B-ox – β -oxidation; DNL – *de novo* lipogenesis; FA-CoA – fatty acyl CoA; TCA/ETC – tricarboxylic acid/electron transport chain; TG – triglyceride; VLDL – very low density lipoprotein. Liver image, “Creative Commons Liver 2”, by Smart Servier Medical Art, licensed under CC BY 3.0, desaturated from original.

Figure 1: Sex differences in skeletal muscle lipid metabolism. Bolded text denotes metabolic pathways that are elevated in women as compared with men. β -ox – β -oxidation; ATGL – adipose triglyceride lipase; CPT-1 – carnitine palmitoyl transferase 1; CPT-2 – carnitine palmitoyl transferase 2; Cyt – cytosol; ETC – electron transport chain; FABP – fatty acid binding protein; FAcarn – fatty acyl – carnitine; FA-CoA – fatty acyl CoA; FAT/CD36 – fatty acid transporter/CD36; FATP – fatty acid transport protein; HSL – hormone sensitive lipase; IMCL – intramyocellular lipid; mtGPAT – mitochondrial glycerol-3-phosphate acyltransferase; PLIN5 – perilipin 5; SREBP-1c – sterol regulatory element-binding protein 1c; TAGH – triacylglycerol hydrolase; TFP – trifunctional protein; VLCAD – very long chain acyl CoA dehydrogenase.

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Figure 2: Sex differences in hepatic lipid metabolism. B-ox – β -oxidation; DNL – de novo lipogenesis; FA-CoA – fatty acyl CoA; TCA/ETC – tricarboxylic acid/electron transport chain; TG – triglyceride; VLDL – very low density lipoprotein. Liver image, "Creative Commons Liver 2", by Smart Servier Medical Art, licensed under CC BY 3.0, desaturated from original.

261x134mm (300 x 300 DPI)