The Investigation of Intestinal/Muscle Lipid Metabolism and Meat Quality in Low Birth Weight Swine

by

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ABSTRACT

Metabolic syndrome and obesity-related complications such as insulin resistance (IR), type II diabetes (T2D) and cardiovascular disease (CVD) have remained a critical worldwide health issue. A previously published study by our group revealed that low birth weight (LBW) piglets exhibited IR and developed preferential intestinal lipid absorption, hypertriglyceridemia and muscle steatosis when fed a high fat (HF) diet compared to normal birth weight (NBW) piglets. For my thesis, we subsequently hypothesized that fatty acid transporters such as CD36, FATP4, FABP2 and/or VAMP7 could potentially explain the development of these observations. Additionally, LBW swine are not often valued in the industry and/or removed from the market due to reduced growth rate and meat quality. We have identified that preferential lipid deposition in muscle tissue may yield a human health benefit by increasing the proportion of beneficial fatty acids for meat consumption. At the same time, dairy-derived fatty acids have been shown to provide beneficial effect on IR, which can be potentially incorporated into pork muscle. Dairy ingredients (lactose and whey protein) can also potentially enhance growth performance in weaning piglets. Therefore, the overall aim of this thesis was to investigate the impact of LBW and dairy intake on intestinal and/or muscle lipid metabolism, as well as on postnatal growth, meat quality and carcass traits. At 5-weeks of age, NBW and LBW piglets consumed a 1-week transition diet then were randomly assigned to three experimental diets: 1-chow diet, 2-HF diet or 3-isocaloric HF diet supplemented with 3 servings per 2000kcal/day of full fat dairy products (HF+Dairy). At 12-weeks of age, piglets were euthanized, and carcass, fasting plasma, muscle tissues (*longissimus* muscle and *biceps femoris*) and jejunum mucosal scrapings were collected. Results revealed that LBW-HF swine exhibited an early sign of IR (fasting glucose, $p<0.05$; fasting insulin, $p=0.091$; HOMA-IR, $p=0.086$) compared to NBW-Chow, which were

ameliorated by dairy intake. Muscle triglyceride content in the LBW-HF group was significantly higher than NBW-Chow group ($p<0.05$). Dairy intake increased myristic acid (C14:0), DPA (C22:5n3) and DHA (C22:6n3) relative to HF feeding alone ($p<0.05$) in LBW swine. All HF fed LBW swine (regardless of dairy intake) exhibited an upregulation of CD36 expression (but not FABP2) compared with NBW littermates in both small intestine and muscle ($p<0.05$). Interestingly, LBW swine did not exhibit significant differences in regards to growth performance or overall meat quality (i.e. tenderness, pH, color and carcass yield) compared to NBW counterparts. However, dairy intake significantly increased Canadian Lean Yield % in LBW-HF+Dairy swine compared to LBW-HF and NBW-HF swine ($p<0.05$).

In summary, findings from this thesis provide new evidence on intestinal/muscle lipid metabolism and meat/carcass quality parameters. We have demonstrated that increased intestinal lipid absorption and preferential muscle lipid deposition in LBW swine can be potentially explained by the upregulation of CD36. Increasing dairy intake can potentially add value to both pork (increasing long-chain PUFA content) and carcass (increasing Canadian lean yield %) quality. Our findings also suggest that LBW pork quality may not be necessarily impaired, which is dependent on LBW phenotype and feeding practice.

PREFACE

Enclosed in this thesis is the original work of Kun Wang with contributions described below:

Chapter 3 has been submitted to *Frontiers in Veterinary Science* for publication (under review as of September 2022) as *"Kun Wang, Yongbo She, Rabban Mangat, Alexander Makarowski, Bimol C. Roy, Heather L. Bruce, Michael K. Dyck, Caroline Richard and Spencer D. Proctor. Preferential deposition of dairy derived fatty acids in muscle tissue is partially due to the upregulation of CD36 in a low-birth-weight swine model."*

Assistance was appreciated and acknowledged from students and staff from the Proctor, Richard and Bruce labs and the Swine Research and Technology Centre (SRTC). Sharon Sokolik, Yongbo She, Rabban Mangat, Alexander Makarowski and SRTC staff assisted in animal housing, surgeries, and/or sample collections along with Kun Wang. Experimental design, data analysis and writing were completed by Kun Wang.

Chapter 4 is an adaptation of a research paper led by Bimol C. Roy. The manuscript is in preparation and will be submitted to *Canadian Journal of Animal Science* as *"Bimol C. Roy, Patience Coleman, Meghan Markowsky, Kun Wang, Yongbo She, Caroline Richard, Spencer Proctor and Heather L. Bruce. Muscle fiber, connective tissue and meat quality characteristics of pork from low-birth-weight pigs as affected by diet-induced increased fat absorption and preferential muscle marbling."*

Assistance was appreciated and acknowledged from students and staff from the Proctor, Richard and Bruce labs, the SRTC and the Agri-Food Discovery Place who assisted in animal housing, surgeries, and/or sample collections.

The research experiments containing animal use described in this thesis were approved by the University of Alberta Animal Care and Use Committee for Livestock (ACUC) and the Canadian

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"Inspiration is a guest that does not willingly visit the lazy."

— Pyotr Ilyich Tchaikovsky

Dedication

To my beloved family, the symbol of love and support

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I am deeply grateful to all professors, students and staff at the University of Alberta who were involved in this project. It would be impossible to complete this thesis without you all. I would like to express my sincerest gratitude to my supervisors Drs. Spencer Proctor and Caroline Richard for your guidance, encouragement, criticism and support throughout my MSc program. I joined the Proctor lab during the last year of my undergraduate study. I met Dr. Richard in 2019 as a trainee before I started my MSc program. Thank you both for guiding me to the field of metabolic diseases and being my mentors throughout the academic journey. You always inspire me to learn more.

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Last but not the least, I would like to thank my mom, dad, Miffy and my band JZ Cocktail for being my source of power. Thank you all for untiredly encouraging me with endless support.

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ACC	Acetyl-CoA carboxylase
ACLY	ATP citrate lyase
ALA	Alpha-linolenic acid
AMPK	Adenosine monophosphate-activated protein kinase
AOAC	Association of Official Agricultural Chemists
ApoB100	Apolipoprotein B100
ApoB48	Apolipoprotein B48
AS160	Akt substrate of 160 kDa
B0AT1	Sodium-dependent neutral amino acid transporter
CD36	Cluster of differentiation 36, also known as scavenger receptor class B protein (SR-B2)
CE	Cholesterol ester
ChoRE	Carbohydrate responsive element
ChREBP	Carbohydrate responsive element binding protein
CI	Confidence interval
CM	Chylomicron
CPT1	Carnitine palmitoyl acyl-CoA transferase 1
CVD	Cardiovascular disease
DAG	Diacylglycerol
DFD	Dark, firm, dry
DGAT	Diacylglycerol acyltransferase
DHA	Docosahexaenoic acid
DIO	Diet induced obesity
DPA	Docosapentaenoic acid
EAAC1	Excitatory amino acid transporter 1
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ERK	Extracellular-signal-regulated kinase
FABP1	Fatty acid binding protein 1
FABP2	Fatty acid binding protein 2

FABP4	Fatty acid binding protein 4
FASN	Fatty acid synthase
FATP1	Fatty acid transport protein 1
FATP4	Fatty acid transport protein 4
GLUT2	Glucose transporter 2
HADH	Hydroxyacyl-CoA dehydrogenase
HBW	Heavy birth weight
HDL	High-density lipoprotein
HF	High fat diet
HF+Dairy	High fat diet supplemented with full-fat dairy products
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IDL	Intermediate-density lipoprotein
IGF	Insulin-like growth factor
IGFBP-3	Insulin-like growth factor binding protein 3
IR	Insulin resistance
IRS-1	Insulin receptor substrate-1
LBW	Low birth weight
LCAD	Long-chain acyl-CoA dehydrogenase
LCFA	Long-chain fatty acid
LDL	Low-density lipoprotein
LDLr	LDL receptor
L-FABP	L-type fatty acid binding protein
LPL	Lipoprotein lipase
MAG	Monoacylglycerol
mATPase	Myofibrillar adenosine triphosphatase
MGAT	Monoacylglycerol acyltransferase
mRNA	Messenger RNA
mTOR	The mammalian target of rapamycin
MUFA	Monounsaturated fatty acid
NADH-TR	Nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase

NAFLD	Non-alcoholic fatty liver disease
NBW	Normal birth weight
NZO	New Zealand Obesity strain
PCTV	Pre-chylomicron transport vesicle
PepT1	Peptide transporter 1
PL	Phospholipid
PPARα	Peroxisome proliferator-activated receptor-alpha
PPARβ	Peroxisome proliferator-activated receptor-beta
PPARγ	Peroxisome proliferator-activated receptor-gamma
PSE	Pale, soft, exudative
PUFA	Polyunsaturated fatty acid
PVDF	Polyvinylidene difluoride
RER	Rough endoplasmic reticulum
RIPA	Radio-immuno-precipitation assay
SCD1	Stearoyl-CoA desaturase-1
SER	Smooth endoplasmic reticulum
SFA	Saturated fatty acid
SGLT1	Sodium-glucose co-transporter 1
SRE	Sterol regulatory element
SREBP-1c	Sterol regulatory element-binding protein 1c
SRTC	Swine Research Technology Centre
T2D	Type II diabetes
TC	Total cholesterol
TCA	Tricarboxylic acid cycle
TG	Triglyceride
TRL	Triglyceride-rich lipoprotein
VAMP7	Vesicle-associated membrane protein 7
VLDL	Very-low-density lipoprotein
VMH	Ventromedial hypothalamus
WAT	White adipose tissue
WHC	Water-holding capacity

CHAPTER 1. Literature review

1.1 Background review

Over the past decade, we have seen the prevalence of cardiovascular disease (CVD), type II diabetes (T2D) and metabolic syndrome continue to rise in many developed countries (World Health Organization, 2021). Metabolic syndrome refers to a cluster of factors that increases the risk for CVD and T2D such as 1. abdominal obesity, 2. hypertension, 3. fasting hyperglycemia, 4. hypertriglyceridemia and 5. low HDL cholesterol. Metabolic syndrome is diagnosed when an individual has 3 or more of these factors (Huang, 2009). In Canada, around 90% of adults have at least one risk factor for CVD with 10% being diagnosed with ischemic heart disease or stroke (Public Health Agency of Canada, 2021; Heart and Stroke Foundation of Canada, 2021). Moreover, 1 in 300 children and youth or 1 in 10 adults have also been diagnosed with diabetes (Public Health Agency of Canada, 2021). The increased prevalence of obesity along with poor dietary patterns (i.e. western-type diet) is a significant contributor to the development of CVD and T2D in our society (Sala and Pontiroli, 2020). In this regard, it is well known that obesity is associated with insulin resistance (IR), which leads to perturbations in glucose and/or lipid metabolism (Kahn et al., 2006; Powell-Wiley et al., 2021). Western diets that are high in saturated fatty acids (SFA), refined sugar and low in fiber are associated with both dysglycemia and dyslipidemia, further exacerbating CVD and T2D risk (Lichtenstein and Schwab, 2000; Haley et al., 2017; Muller et al., 2021).

Lipid metabolism involves the breakdown, absorption, synthesis and transport of lipids. Dyslipidemias refer to elevated total or low-density lipoprotein (LDL) cholesterol levels, reduced high-density lipoprotein (HDL) cholesterol level, and/or elevated triglyceride (TG) levels (Kopin and Lowenstein, 2017). Lipids can be synthesized endogenously by the liver via *de novo*

lipogenesis. The liver secretes TG-rich very-low-density lipoprotein (VLDL) to transport lipids to tissues (Genest, 2003). Cholesterol-rich (and small dense) LDL particles are formed by the hydrolysis of TG in VLDL and have become one of the most widely used risk indices for CVD (Genest, 2003). Notably, researchers have also learned that intestinal-derived remnant lipoproteins also play a key role in the progression of CVD (Proctor et al., 2002; Mangat et al., 2007; Vine et al., 2020). The intestine secretes large TG-rich chylomicrons that serve to absorb fat and lipid soluble nutrients. Once metabolized, smaller chylomicron remnant lipoproteins remain in circulation and can contribute to plaque development (Dash et al., 2015). In the context of IR, there can be an increased production of both chylomicrons and the VLDL, resulting in hypertriglyceridemia (Dash et al., 2015). This in turn can also lead to a reduced clearance of LDL and remnant particles, further contributing to the CVD risk (Dash et al., 2015). On the other hand, hypertriglyceridemia has been associated with ectopic lipid accumulation and lipotoxicity (Defronzo, 2009). In muscle tissue, ectopic lipid accumulation has been linked to reduced mitochondrial function; whereas in the pancreas, lipotoxicity is associated with impaired beta cell function and contributes to the development of T2D (Defronzo, 2009; Schrauwen, 2007).

However, there still remains a lack of clarity about the molecular mechanisms related to the increased intestinal lipid absorption and muscle steatosis during conditions of IR and is the general focus of this thesis. To understand the importance of the metabolic adaptation as it relates to intestinal lipid metabolism and/or muscle lipid deposition, this literature review will cover topics related to three main areas 1. lipid metabolism in the liver, small intestine and muscle, 2. the role of diet on metabolic diseases, and 3. The pre-clinical animal model (swine) in metabolic studies.

1.2 Physiology of lipid metabolism

Nutrients from the diet are absorbed by the intestine and transported to liver and peripheral tissues via blood circulation. Dietary fat undergoes hydrolysis in the lumen of the intestine, then hydrolyzed lipid products are absorbed by enterocytes via passive diffusion or facilitated by fatty acid transporters (depending on the size of the fatty acids) (Dash et al., 2015). TG is reassembled in the enterocyte and packaged into large lipoprotein particles, namely chylomicrons (Dash et al., 2015). Nascent chylomicrons are secreted into lymph and then enter bloodstream via the thoracic duct to transport lipid-soluble nutrients. Both liver and intestine are the two primary organs responsible for lipid homeostasis, namely production, secretion and/or clearance of lipoproteins (Dash et al., 2015).

Lipoproteins are a bi-layer particles with the outer layer containing the amphipathic phospholipids (PL), free cholesterol and apolipoproteins; the central hydrophobic core of non-polar lipids primarily consists of cholesterol esters (CE) and TG (**Figure 1-1**) (Zipes et al., 2018). Lipoproteins can be classified according to different densities, size and the composition (**Figure 1-2**) (Zipes et al., 2018).

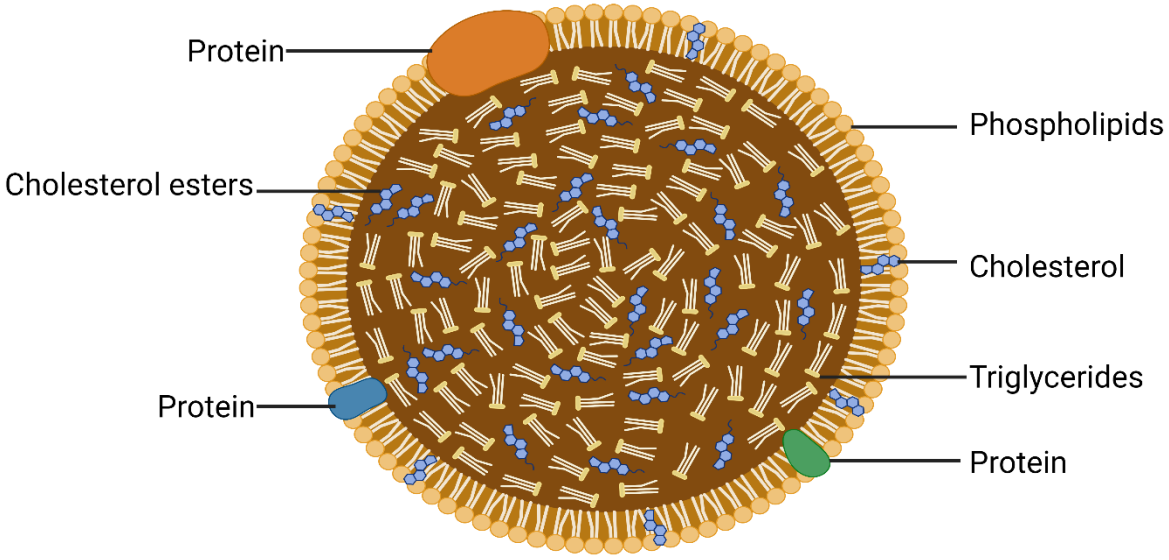

Figure 1-1. The structure of lipoprotein (chylomicron). Created with BioRender. Adapted from Zipes et al., 2018.

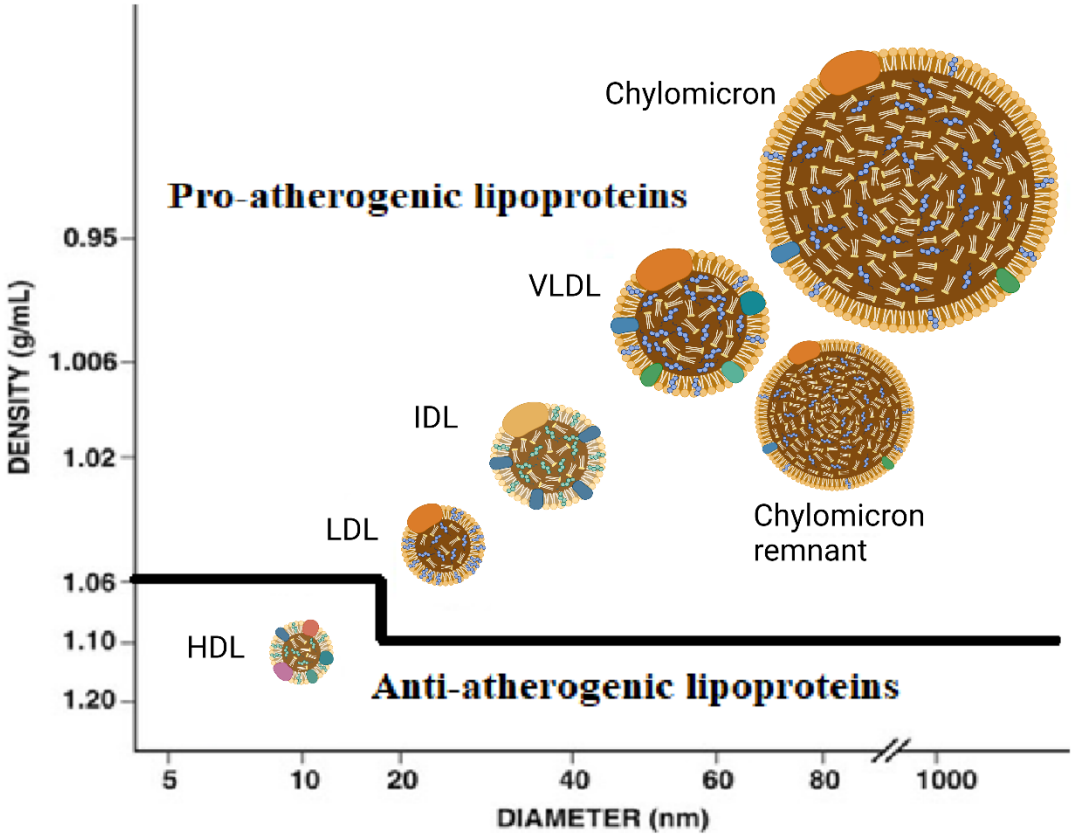

Figure 1-2. The diameter and the density of lipoproteins. HDL: high-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; VLDL: very-low-density lipoprotein. Created with BioRender. Adapted from Zipes et al., 2018.

1.2.1 Hepatic lipid metabolism

Hepatic lipid metabolism contains three main elements: 1. fatty acid uptake and *de novo* lipogenesis; 2. lipid storage (including TG synthesis and the formation of lipid droplets); 3. lipid utilization (beta-oxidation, as well as the assembly and secretion of VLDL) (Enjoji et al., 2016). Fatty acid uptake in hepatocytes is primarily from either esterified fatty acids from the hydrolysis of dietary TG (transported by intestinal-derived chylomicrons during the postprandial state), or non-esterified free fatty acids (lipolysis of adipose tissue, then transported by albumin during the

fasting state) (Enjoji et al., 2016). Glucose can be a source for lipogenesis as well, and in human, excess glucose undergoes a series of conversions to form acetyl-CoA (glycolysis and tricarboxylic acid cycle, TCA) (Ameer et al., 2014). Acetyl-CoA is converted into malonyl-CoA by acetyl-CoA carboxylase (ACC), which is a key substrate in *de novo* lipogenesis. Fatty acid synthase (FASN, the rate-limiting enzyme) acts on this substrate to catalyze the synthesis of palmitic acid (C16:0) (Ameer et al., 2014). After a series of elongation or desaturation reaction, longer chain fatty acids are formed (Ameer et al., 2014).

Dietary patterns play an important role in the regulation of this lipogenic pathway (Ameer et al., 2014). High-carbohydrate diets (especially fructose) have been shown to increase hepatic *de novo* lipogenesis, as well as the synthesis and secretion of VLDL, resulting in hypertriglyceridemia and potentially non-alcoholic fatty liver disease (NAFLD) (Bjorntorp and Sjostrom, 1978; Ameer et al., 2014). Simple sugars activate the expression of carbohydrate responsive element binding protein (ChREBP) in hepatocytes, which lead to the translocation of this transcription factor from cytoplasm to nucleus, binding to carbohydrate responsive elements (ChoREs) to activate the expression of lipogenic genes (**Figure 1-3**) (Heidenreich et al., 2020). Glucose also stimulates the release of insulin and inhibits the release of glucagon in pancreas. In turn, insulin activates the sterol regulatory element-binding protein 1c (SREBP-1c) in the liver at both the transcriptional level and post-translational level, resulting in the increased expression of lipogenic genes (**Figure 1-3**) (Enjoji et al., 2016).

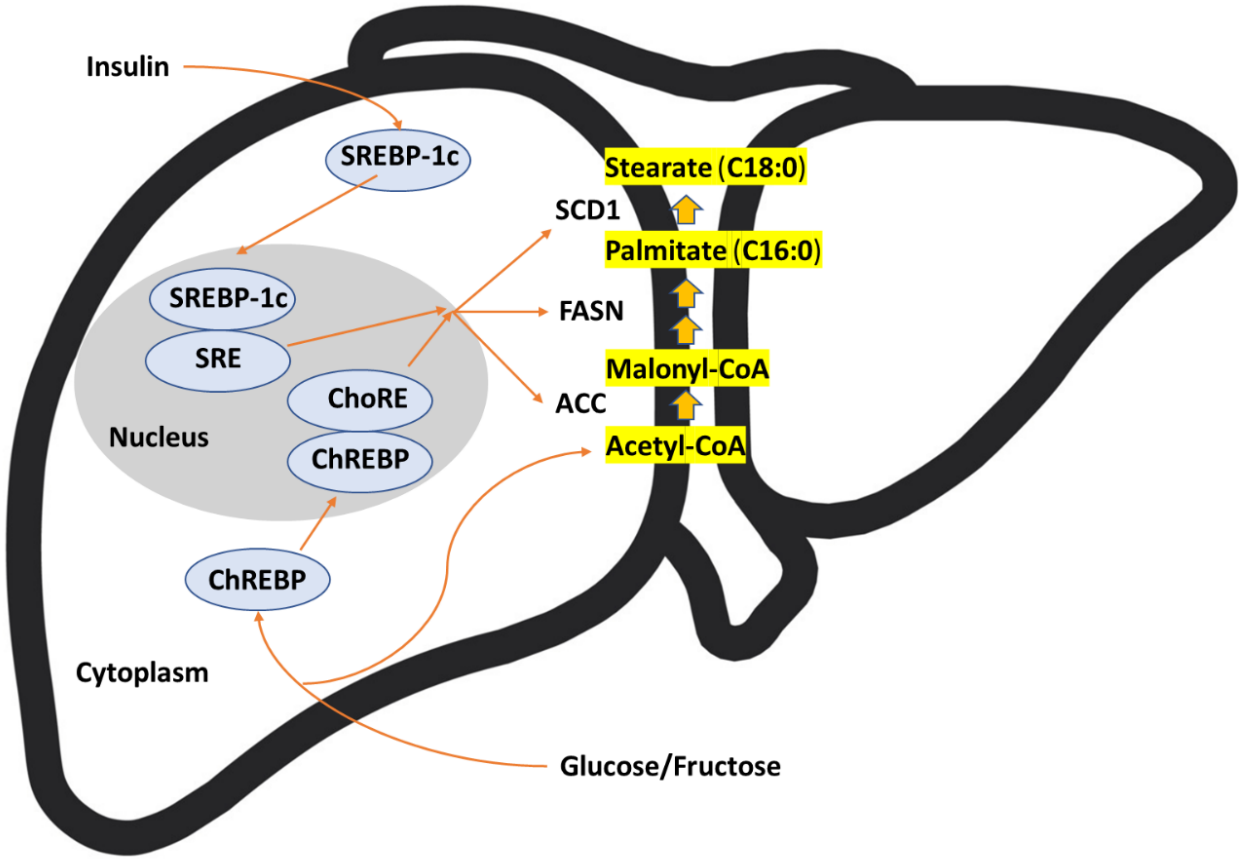

Figure 1-3. Glucose/fructose and insulin induced de novo lipogenesis in liver. Simple sugars intake activates ChREBP, which binds to ChoRE in the nucleus to activate lipogenic genes. Insulin activates SREBP-1c, which binds to lipogenic gene promoter regions at SRE in the nucleus. ChREBP: carbohydrate responsive element binding protein; ChoRE: carbohydrate responsive elements; SREBP-1c: sterol regulatory element-binding protein 1c; SRE: sterol regulatory element; SCD1: stearoyl-CoA desaturase-1; FASN: fatty acid synthase; ACC: acetyl-CoA carboxylase. Adapted from Tappy and Le (2010) and Dif et al. (2006).

In human hepatocytes, TG is synthesized from diacylglycerol (DAG) and fatty acyl-CoA by membrane-bound enzymes acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) and DGAT2 (Enjoji et al., 2016). Synthesized TG is either stored in cytoplasmic lipid droplets (mediated by DGAT 2) or participates in the assembly and secretion of VLDL (mediated by DGAT 1) (Enjoji et al., 2016). Under normal conditions, the liver is typically not a lipid storage site with very little TG (TG content <5%) (Enjoji et al., 2016). However, in patients with obesity, excess lipid storage can occur, namely hepatic steatosis (Enjoji et al., 2016). Hepatic steatosis can result in aberrant gluconeogenesis and further promote the development of T2D (Minehira and Gual, 2017).

Beta-oxidation of fatty acids refers to the process of the breakdown of fatty acids into acetyl-CoA units. Acetyl-CoA further undergoes TCA cycle or is converted to ketone bodies to produce energy (ATP). The oxidation of short, medium or long-chain fatty acid occurs in mitochondria, while the oxidation of toxic or very long-chain fatty acid occurs in peroxisomes (Berlanga et al., 2014). Carnitine palmitoyl acyl-CoA transferase 1 (CPT1) is the rate-limiting enzyme for mitochondrial beta-oxidation, which is involved in the transport of fatty acids from cytoplasm to mitochondria (Enjoji et al., 2016). The activity of this enzyme is inhibited by malonyl-CoA, which is the main substrate in *de novo* lipogenesis in the energy-sufficient state (**Figure 1-4**) (Enjoji et al., 2016).

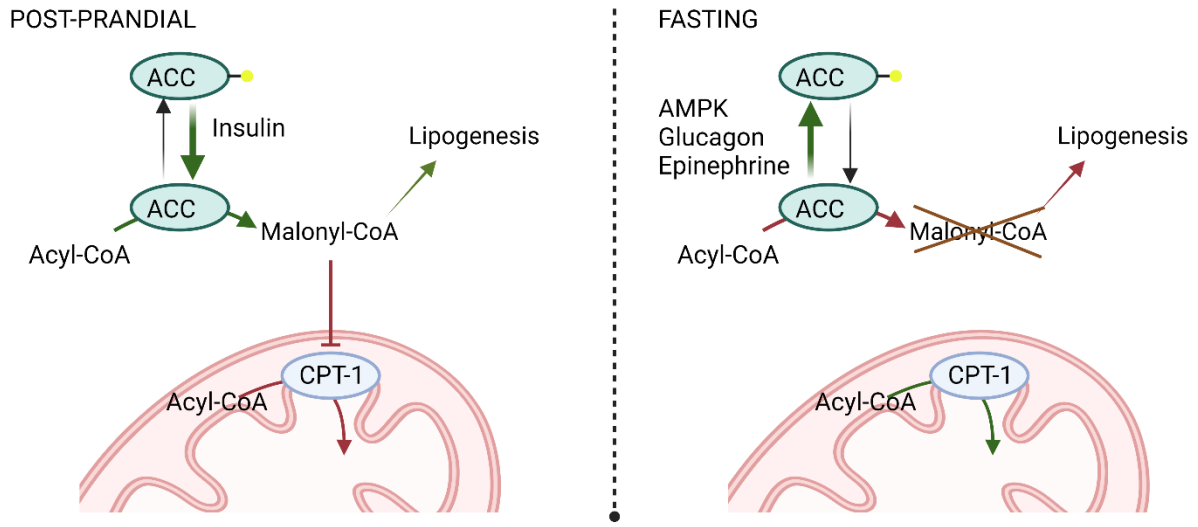

Figure 1-4. The regulatory role of Malonyl-CoA on lipogenesis and fatty acid oxidation. At postprandial state, insulin activates ACC by promoting the dephosphorylation. ACC converts acetyl-CoA to malonyl-CoA. Malonyl-CoA inhibits beta-oxidation by inhibiting CPT-1 activity. At fasting state, catabolic hormones (i.e. glucagon, AMPK, epinephrine) inactivates ACC through the phosphorylation. CPT-1 activity is increased, promoting fatty acid oxidation. ACC: acetyl-CoA carboxylase; AMPK: adenosine monophosphate-activated protein kinase; CPT-1: carnitine palmitoyl acyl-CoA transferase 1. Created using BioRender. Adapted from Longo et al. (2019).

The key regulator of beta-oxidation is peroxisome proliferator-activated receptor alpha (PPARα), which upregulates the genes involved in the oxidation of fatty acids such as hydroxyacyl-CoA dehydrogenase (HADH), long-chain acyl-CoA dehydrogenase (LCAD), and CPT1 in liver (Enjoji et al., 2016; Mandard et al., 2004; Harano et al., 2006). PPARα also inhibits the lipogenesis or lipid storage in liver; the activation of this transcriptional factor by fenofibrate has been shown to effectively alleviate hepatic steatosis or NAFLD (Souza-Mello, 2015). When the supply of lipids to the liver is adequate, hepatic VLDL assembly is increased

(Enjoji et al., 2016). Unlike intestinal-derived chylomicrons, VLDLs contain a full-length ApoB100 molecule. ApoB100 is the primary structural protein for VLDL assembly. The synthesis rate of ApoB100 determines the overall rate of VLDL assembly (and secretion) (Enjoji et al., 2016). Microsomal triglyceride transfer protein (MTTP) is one of the primary rate-limiting enzymes for the assembly of VLDL, responsible for the translocation of ApoB100, the incorporation of TG to ApoB100, and the secretion of VLDL (Enjoji et al., 2016). The liver secrets VLDL into blood circulation to transport lipids to peripheral tissues. The nascent VLDL contains ApoB100, ApoC-1, ApoE, cholesterol, CE, as well as TG (Enjoji et al., 2016). VLDL further receives ApoC-II and extra ApoE from HDL from the circulation (Shelness and Sellers, 2001). ApoC-II on the lipoprotein surface activates lipoprotein lipase (LPL) to release lipid from VLDL (Shelness and Sellers, 2001). VLDL is rapidly converted to intermediate-density lipoprotein (IDL) following loss and hydrolysis of TGs from the original VLDL particle (reducing its overall size and increasing particle density). IDL is further converted to LDL by hepatic triglyceride lipase in the liver, mediated by ApoE (Shelness and Sellers, 2001). LDL is a well-known classic risk factor of CVD, however interestingly, intestinal-derived chylomicrons and their remnant lipoproteins are also known to play an important role in the development of atherosclerosis.

1.2.2 Intestinal lipid metabolism

The small intestine contains plentiful intestinal villi for the absorption of nutrients (Ko et al., 2020). Dietary lipids mainly consist of TG, PL and CE. The absorption pathway of lipids is different from other macronutrients, which involves the breakdown of dietary lipids in the lumen, the transport of fatty acids and monoglycerides in enterocytes, and finally the re-esterification of digested products into complex lipids within enterocytes (Ko et al., 2020).

Eventually, most of intracellular TGs are incorporated into chylomicrons, which deliver dietary lipids throughout the body (Ko et al., 2020).

1.2.2.1 Triglyceride absorption and reassembly

Bile acid-containing micelles emulsify dietary lipids to render them soluble in the duodenum and jejunum whereby they are further digested by lipases to yield monoacylglycerol (MAG) and free fatty acids (Dash et al., 2015). The absorption of MAG and free fatty acid occurs in part by passive diffusion via a concentration gradient (Dash et al., 2015). The absorption of long-chain fatty acid (LCFA) is facilitated by multiple transporters such as fatty acid binding protein 2 (FABP2), fatty acid transport protein 4 (FATP4) and cluster of differentiation 36 (CD36, also known as scavenger receptor class B protein, SR-B2) (Stremmel, 1988; Stahl et al., 1999; Nauli et al., 2006). The following table (**Table 1-1**) summarized tissue distributions of fatty acid transporters in humans and/or mice.

Table 1-1. Distributions of fatty acid transporters in tissues in humans and/or mice.

Types	Small intestine	Heart	Liver	Skeletal muscle	Brain	WAT
FABP1	√√	**x**	√√	**x**	**x**	**x**
FABP2	√√	**x**	**x**	**x**	**x**	**x**
FABP4	**x**	~	**x**	**x**	**x**	√√
CD36	√√	√√	√	√√	√	√√
FATP1	**x**	√√	**x**	√√	√	√√
FATP4	√√	~	√	~	√	~

FABP: fatty acid binding protein; FATP: fatty acid transport protein; CD36: cluster of differentiation 36; WAT: white adipose tissue; √√: high expression; √: expressed; ~: weak expression; **x**: not expressed. Expressions were assessed by a combination of northern blot, western blot, RNA sequencing and in situ hybridization. Adapted from Stahl et al. (2001), Dobri et al. (2021), McDermott et al. (2021), Glatz and Luiken (2017), Auinger et al. (2010) and Uhlen et al., (2015).

The relationship between SREBP-1c-induced lipogenesis and CD36 in liver was underscored by a study demonstrating that mice lacking hepatic CD36 expression exhibited a significant downregulation of SREBP-1c and the downstream lipogenic enzymes such as FASN, ACC or ATP citrate lyase (ACLY) (Zeng et al., 2022). Overexpression of CD36 in liver can activate *de novo* lipogenesis and the formation of lipid droplets, suggesting that CD36 may also promote the hepatic lipogenesis (Zeng et al., 2022). Interestingly in the small intestine, the absence of some of these transporters (at least in humans) does not necessarily affect LCFA absorption. Mice treated with FATP4 inhibitors did not show any impairment to fat absorption,

while CD36 knockout mice exhibited fat malabsorption in the proximal intestine (but not in the distal gut) with decreased chylomicron secretion (Blackburn et al., 2006; Nassir et al., 2007). In contrast, humans with CD36 deficiency have been shown to have hypertriglyceridemia, IR and hypertension due to increased level of chylomicron remnants with no notable lipid malabsorption (Masuda et al., 2009). After entering enterocytes, fatty acid is converted to fatty acyl-CoA (mediated by FATP4). Fatty acyl-CoA is combined with MAG to form DAG, catalyzed by monoacylglycerol acyltransferase (MGAT) (Dash et al., 2015). DAG is further acylated to form TG, catalyzed by DGAT (Dash et al., 2015). TG can also be synthesized from dietary carbohydrates via *de novo* lipogenesis in enterocytes, which accounts for a quarter of total TG synthesis (Hoffman et al., 2019; Dash et al., 2015). The small intestine is not a long-term lipid storage site, and lipids will eventually be mobilized and packaged into chylomicrons.

1.2.2.2 Chylomicron assembly, secretion and clearance

Intestinal-derived chylomicrons are TG-rich particles that are responsible for the transport of dietary TG and fat-soluble substances to peripheral tissues and liver (Bayly, 2014). These particles contain a variety of apolipoproteins such as ApoA-I, ApoA-II, ApoA-IV, ApoA-V, ApoC-II, ApoC-III, ApoE, as well as the principal structural protein ApoB-48 (Bayly, 2014). ApoB-48 is essential for the chylomicron assembly (equivalent to ApoB100 for VLDL).

Each chylomicron particle contains only one ApoB-48, and in the research context can provide an indication of the number of particles/concentration in circulation. Chylomicron assembly starts at the rough endoplasmic reticulum (RER) by incorporating ApoB-48 into a high-dense phospholipid-rich nascent chylomicron particle, facilitated by MTTP (**Figure 1-5**) (Giammanco et al., 2015). The transfer protein MTTP is also responsible for the formation of ApoB-free lipid droplets in the smooth endoplasmic reticulum (SER), which are further

integrated into nascent chylomicron particles, forming pre-chylomicrons (**Figure 1-5**) (Giammanco et al., 2015). Pre-chylomicrons are packaged into a transport vesicle, namely pre-chylomicron transport vesicle (PCTV), which is further transported to *cis*-Golgi from the ER membrane (**Figure 1-5**) (Giammanco et al., 2015).

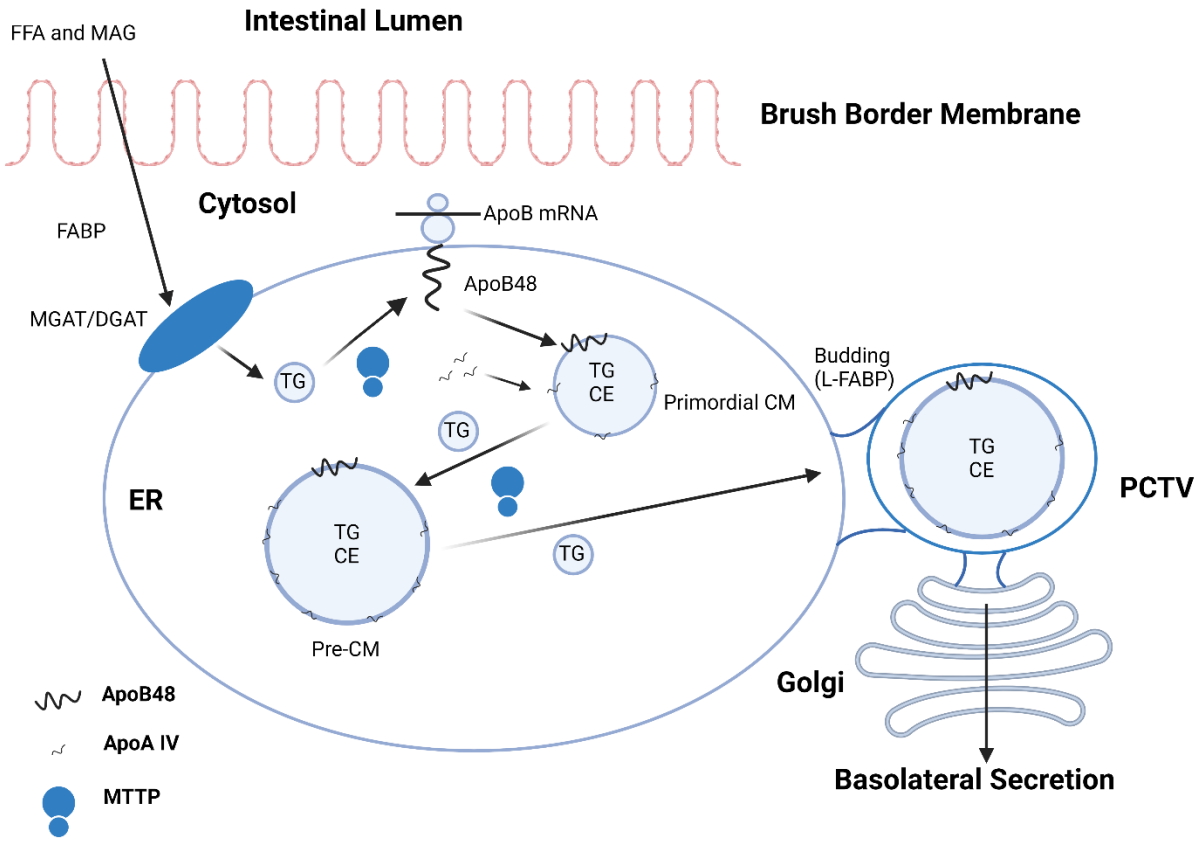

Figure 1-5. The assembly and secretion of chylomicron. Triglyceride (TG) is re-assembled in endoplasmic reticulum (ER) from fatty acid and monoacylglyceride (MAG) by acyl-CoA:monoacylglycerol acyltransferase (MGAT) and acyl-CoA:diacylglycerol acyltransferase (DGAT). Nascent ApoB48 is lipidated by microsomal triglyceride transferase (MTTP) with the addition of cholesterol ester (CE) and other apolipoproteins such as ApoA-IV to form pre-chylomicron (pre-CM). Pre-CM is packaged into a transport vesicle, namely pre-chylomicron transport vesicle (PCTV), which is facilitated by L-type fatty acid binding protein (L-FABP). Pre-CM is processed at the Golgi apparatus to form mature CM, which is released at the basolateral membrane. Created with BioRender. Adapted from Black, 2007.

It has been suggested that L-type fatty acid binding protein (L-FABP, also known as liver fatty acid binding protein or FABP1), CD36 and vesicle-associated membrane protein 7 (VAMP7) are also involved in the formation of PCTV (Cifarelli and Abumrad, 2018; Siddiqi et al., 2010). Mature chylomicrons are formed after pre-chylomicrons are processed at the Golgi apparatus (Giammanco et al., 2015). They leave enterocytes at the basolateral membrane and are secreted into the lymphatic system, eventually entering the blood circulation via the thoracic duct (Bayly, 2014; Hussain et al., 1996). The size of chylomicron can be dependent on the fat content from a dietary meal. A high fat meal may lead to increased TG incorporation into chylomicrons, resulting in larger particle size (Bayly, 2014). Following lipolysis by lipoprotein lipase (LPL), most of TG in chylomicrons are transported to peripheral tissues, resulting in smaller, more dense and cholesterol-rich chylomicron remnants (Bayly, 2014; Dash et al., 2015). Chylomicron remnants are cleared from the circulation in the liver by binding to LDL-receptor or ApoE-mediated heparan sulphate proteoglycans located on the surface of hepatocytes (Dash et al., 2015). In humans, delayed clearance of chylomicron remnants has been associated with atherosclerosis. Our research team has demonstrated that intestinal-derived chylomicron remnants also contribute to the development of atherosclerosis in both clinical studies and a number of pre-clinical models (Proctor and Mamo, 1996; Proctor et al., 2002; Mangat et al., 2007; Vine et al.,2007; Warnakula et al., 2011; Krysa et al., 2020; Vine et al., 2020).

1.2.2.3 Chylomicron remnants in atherosclerosis and cardiovascular disease

Stroke and CVD remain the leading cause of death worldwide resulting in approximately 18 million deaths each year (World Health Organization, 2022). Obesity-related complications such as IR, T2D and dyslipidemia are associated with increased risk of atherosclerosis (Poznyak et al., 2020; Dash et al., 2015). It has been shown that despite traditional lipid lowering

treatments being effective at reducing LDL-cholesterol and TG, a significant residual CVD risk still remains. Numerous studies have also described the significant role of remnant lipoproteins in CVD risk. Indeed, both chylomicrons and VLDL undergo hydrolysis by LPL and form corresponding remnants, and these remnants are cleared by the liver via similar mechanisms (Dash et al., 2015). Under normal conditions, enterocytes are insulin sensitive, and insulin is able to acutely suppress the secretion of chylomicron in enterocytes (as well as the production of VLDL in liver by promoting ApoB degradation) (Dash et al., 2015; Gill and Sattar, 2011). However, during IR or T2D, enterocytes become resistant to the acute suppressive effect of insulin, causing increased chylomicron production (Dash et al., 2015). Interestingly, exogenous chylomicrons and endogenous VLDL compete with each other due to the same metabolic pathway (LPL-mediated hydrolysis), resulting in a delay in the clearance of these particles, the accumulation of triglyceride-rich lipoproteins (TRLs), as well as postprandial hypertriglyceridemia (**Figure 1-6**) (Dash et al., 2015). Lipoprotein remnant particles are smaller, more dense and rich in cholesterol ester. They are more susceptible to oxidation and can become trapped in the artery wall, triggering the formation of macrophage foam cell and pro-inflammation (Liu et al., 2009; Shin et al., 2004; Ting et al., 2007), which is a critical step in the progression of atherosclerosis (**Figure 1-6**) (Mamo et al., 1998; Proctor et al., 2002).

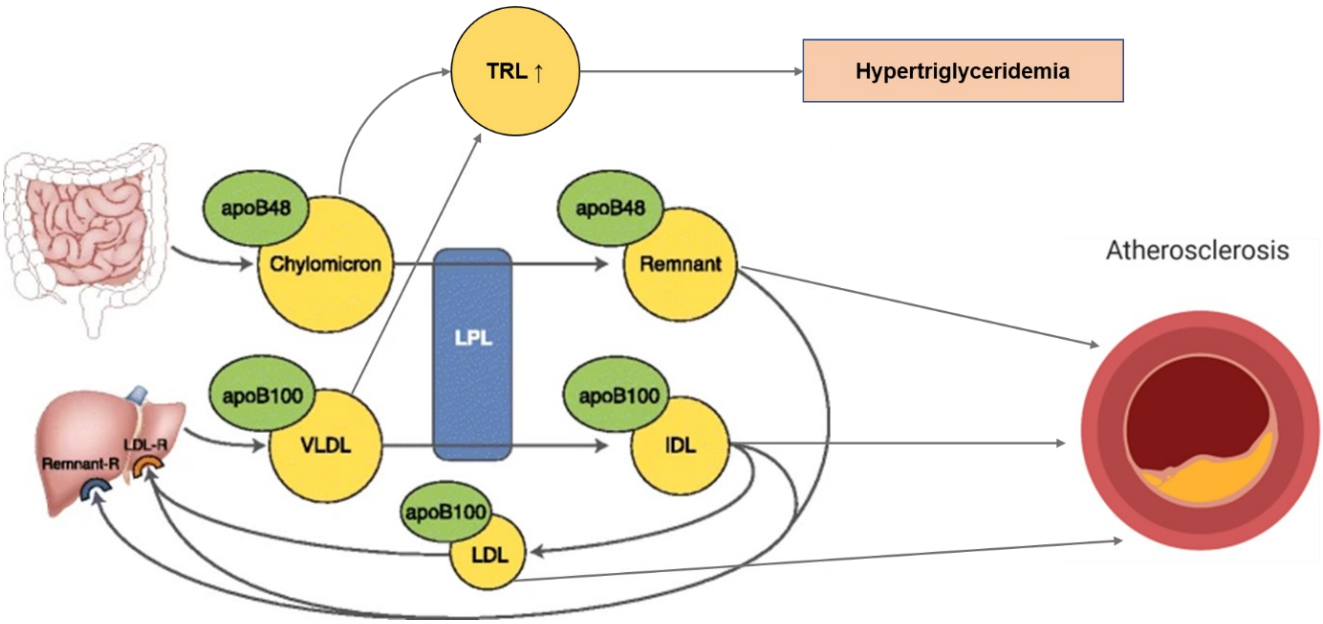

Figure 1-6. The development of hypertriglyceridemia and atherosclerosis. Due to limited LPL availability, the competition between the clearance of VLDL and chylomicron results in the accumulation TRL particles, causing postprandial hypertriglyceridemia. The accumulation of lipoprotein remnants leads to the formation of atherosclerotic plaque. VLDL: very low-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; LPL: lipoprotein lipase; TRL: triglyceride-rich lipoprotein. Adapted from Klop and Cabezas (2012).

1.3 Lipid metabolism during Western diet induced obesity and insulin resistance

The increased prevalence of obesity and IR in developed countries is believed to be a result of sedentary life and obesogenic environments (energy-rich food sources). In individuals with obesity, energy intake is greater than energy expenditure, leading to increased lipid storage in adipose tissue and lipid accumulation at ectopic sites such as muscle (Savage et al., 2007). Here

we will discuss potential consequences and mechanisms of obesity and IR on intestinal lipid absorption and muscle lipid deposition.

1.3.1 Impaired intestinal lipid absorption during insulin resistance

As mentioned previously, hypertriglyceridemia (which is commonly seen in patients with obesity and IR) results from increased production and delayed clearance of chylomicrons and VLDL in the small intestine and liver respectively (Dash et al., 2015). Insulin fails to suppress ApoB-48 lipoprotein production in enterocytes due to reduced phosphorylation of insulin receptor substrate-1 (IRS-1) and decreased expression of Akt (Dash et al., 2015). The extracellular-signal-regulated kinase (ERK) signaling pathway regulates insulin sensitivity in enterocytes. However, increased free fatty acid influx disrupts ERK signaling, further contributing to increased production of chylomicrons (Duez et al., 2008). The impaired clearance of chylomicrons can be disrupted by abnormal activity of LPL caused by IR, reducing the rate of hydrolysis and interfering with receptor mediated uptake (Klop and Cabezas, 2012; Arpon et al., 2019). Additionally, glucose metabolism in the small intestine is disrupted by IR as well. Sodium-glucose co-transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) are major carrier proteins that facilitate glucose absorption in enterocytes (Gromova et al., 2021). Their activities have been found to be increased in diabetic condition, contributing to hyperglycemia (Gromova et al., 2021).

1.3.2 Impaired muscle lipid deposition during insulin resistance

The muscle incorporates fatty acids from the circulation, mediated by specific membrane-bound fatty acid transporters. CD36 is found on both the cell membrane and endosomes in cardiac and skeletal muscle cells (**Figure 1-7**) (Glatz and Luiken, 2017). The reversible translocation of CD36 from endosomes to sarcolemma (the plasma membrane of the muscle

cell), induced by either muscle contraction or the presence of insulin, elevates the uptake of fatty acid (**Figure 1-7**) (Luiken et al., 2002; Luiken et al., 2003). Lipid accumulation in the muscle cell disrupts insulin signaling in individuals with obesity, resulting in IR and contractile dysfunction (Schrauwen, 2007; Glatz and Luiken, 2017). This further impairs contraction-induced and insulin-stimulated CD36 translocation, leading to more lipid deposition in the muscle, which forms a vicious cycle (Glatz and Luiken, 2017). Therefore, CD36 appears to play a critical role in lipid metabolism and the development of IR in the muscle cell.

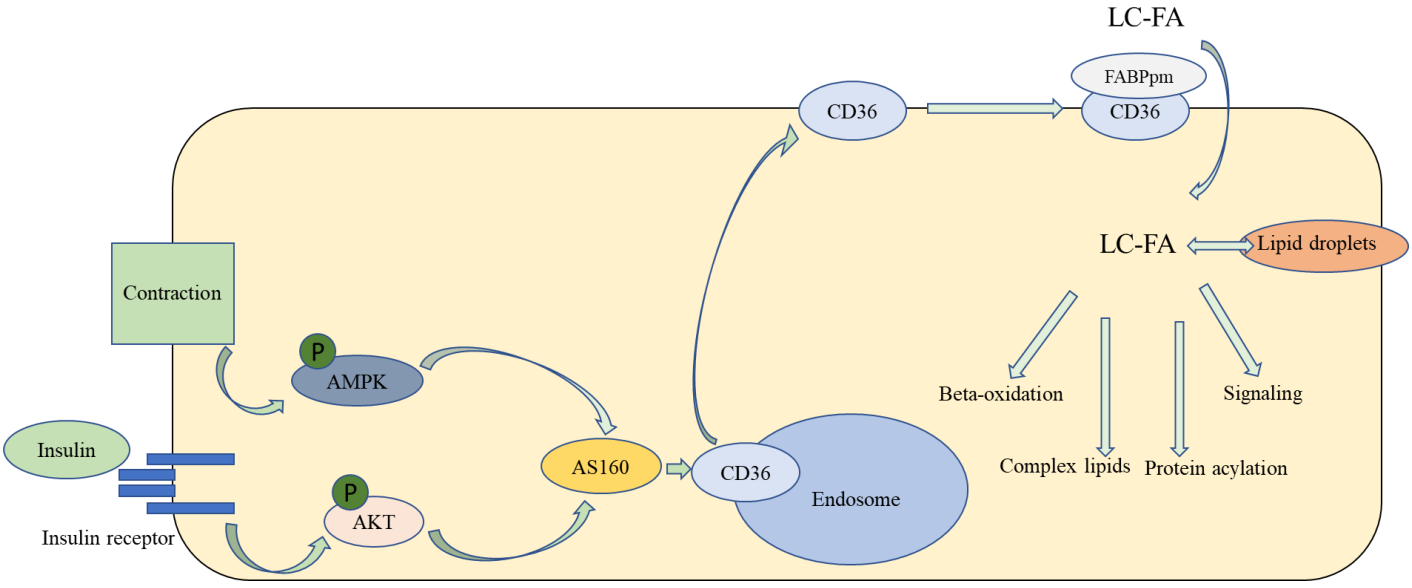

Figure 1-7. The regulatory role of CD36 in LC-FA uptake into cardiac and skeletal myocytes. Muscle contraction or insulin triggers the vesicular transport of CD36 from endosome to sarcolemma (plasma membrane of myocytes), mediated by AMPK and insulin signaling cascades respectively. Contraction and insulin act independently to induce CD36 translocation, but converge at AS160. LC-FA: long-chain fatty acid; AS160: Akt substrate of 160 kDa (a Rab-GTPase activating protein). Adapted from Glatz and Luiken (2017).

Researchers have suggested targeting CD36 to attenuate lipotoxicity and lipid-induced IR, as well as atherosclerosis (Goudriaan et al., 2003; Rekhi et al., 2021). CD36 deficiency in mice has been found to improve insulin sensitivity in muscle but cause IR in liver (Goudriaan et al., 2003). LDL receptor (LDLr) knockout mice fed a high-fat diet that underwent a CD36 inhibitor treatment exhibited reduced atherosclerotic lesion area with lower plasma cholesterol level and reduced pro-inflammatory cytokine levels (TNF-α, IL-1 and MCP-1) (Rekhi et al., 2021). Additionally, PPARs activated by LCFA or their derivatives such as acyl-CoA esters, can regulate the expression of CD36 in a tissue-specific manner. PPARα is expressed in most of tissues such as liver, cardiac and skeletal muscle and kidney. PPARβ is highly expressed in skeletal muscle, whereas PPARγ is mainly expressed in white adipose tissue (Glatz and Luiken, 2017). A transgenic mouse model with the muscle-specific overexpression of PPARα exhibited a 5-fold increase in CD36 gene expression, as well as other genes related to fatty acid uptake (Finck et al., 2005). PPARα deficiency has shown to be protective against diet-induced IR in the skeletal muscle, despite the occurrence of obesity (Finck et al., 2005). Furthermore, glucose uptake also affects muscle lipid content. Glucose is mainly stored as glycogen in muscle under normal conditions. Under conditions of a high-carbohydrate diet, *de novo* lipogenesis activity in muscle is increased, and excess glucose is involved in the synthesis of fatty acid (for storage), potentially contributing to muscle steatosis (Savage et al., 2007).

1.4 The role of diet on metabolic diseases

Western diet and sedentary lifestyle have contributed to a tremendous increase in the prevalence of obesity or obesity-related complications. Modifiable lifestyle factors such as dietary habits have been shown to have protective effects against metabolic diseases, i.e. improving IR, plasma lipid profile as well as inflammatory markers. (Rideout et al., 2013;

Richard et al., 2016; Azarcoya-Barrera et al., 2021). Fat is one of the key macronutrients in a diet. It has been long debated that SFAs are associated with IR, T2D or CVD (Briggs et al., 2017; Risérus et al., 2009). Yet, the restriction of a single nutrient such as SFA without adequate physical activities may have little effect on metabolic syndrome (Castro-Barquero et al.,2020). It is also noteworthy that not all SFAs are considered biologically equal. For example, short chain fatty acids and odd chain fatty acids have been shown to have health benefits in human, and dairy products are an excellent source of these fatty acids.

1.4.1 Dairy-derived fatty acids and other nutrients on metabolic diseases

The association between the consumption of dairy products (including milk, cheese, yogurt and other fermented forms) and the risk of CVD, T2D and metabolic syndrome has been extensively investigated, but conclusions remain debatable. Dairy products have been shown to be positively associated with improvement in insulin sensitivity in either interventional or observational studies (Yoko et al., 2021; Hashemipour et al., 2016; Rideout et al., 2013). Daily dairy consumption has also been associated with a lower risk of glucose intolerance (Yoko et al., 2021; Hashemipour et al., 2016). In addition, more frequent long-term consumption of dairy products (≥4 servings/day for 12 months) has been related to a decreased HOMA-IR score, indicating better insulin sensitivity (Rideout et al., 2013). Notably, there have been discrepancies in a number of public health guidance policies regarding the recommendation of dairy (and/or full fat dairy) intake in relation to health. For example, the latest Canada Food Guide in 2019 actually withdrew the recommendation on dairy consumption as a food group (especially full-fat version) due to the concern about SFA (Health Canada, 2019). Dietary guidelines in the US also only recommends low-fat or fat-free milk or yogurt (USDA, 2020). Indeed, low fat dairy

products contain less SFA and have been associated with lower risk of hypertension and IR (Lordan et al., 2018; Drouin-Chartier et al., 2016).

SFAs have been linked to poor health outcomes, by increasing the risk of CVD and T2D (Briggs et al., 2017; Risérus et al., 2009). Yet, it is also noteworthy that not all SFA have the same metabolic fate. For instance, ruminant-derived dairy products contain a diverse fatty acid profile, which consists of 8%~22% medium chain fatty acids (C6:0 to C12:0), 22%~35% palmitic acid (C16:0), 20%~30% oleic acid (C18:1n9), as well as polyunsaturated fatty acids (PUFA) and unique odd chain fatty acids (Abdullah et al., 2015). Despite the deleterious effect of palmitic acid (C16:0) on insulin sensitivity, both butyric acid (C4:0) and myristic acid (C14:0) have shown a beneficial effect on glycemic control in mice (Amine et al., 2021; Gao et al., 2009; Takato et al., 2017). In particular, short chain fatty acids can also ameliorate ectopic fat deposition in muscle by decreasing the mRNA expression of CD36, as well as decreasing *de novo* lipogenesis in liver by downregulating mRNA expressions of *ACC*, *FASN* and *SREBP-1C* (Zhou et al., 2021). Odd-chain fatty acids such as pentadecanoic acid (C15:0) and margaric acid (C17:0) have also been associated with the lower risk of T2D and total mortality (Zhuang et al., 2019; Imamura et al., 2018). In addition to dairy-derived fatty acids, another nutrient in dairy that can improve IR is the fat-soluble vitamin K2, which is synthesized by bacteria during the fermentation of cheese products (Struijk et al., 2013). The dietary intake of menaquinones (vitamin K2) has been found to be associated with a reduced risk of T2D (Beulens et al., 2010). For more detail regarding beneficial effects of dairy products on human health, please refer to **Appendix A** (a mini-review paper submitted to *Journal of Nutritional Science*).

On the other hand, there have been an equal number of studies that have reported no positive association between dairy consumption and IR, leading to a heavy debate around the

beneficial effect of dairy intake on insulin sensitivity (Benatar et al., 2014; Drouin-Chartier et al., 2016). Indeed, the form of dairy products included (i.e. low-fat and/or full-fat), the amount consumed, the length of the intervention, as well as the number of participants may explain some of these discrepancies among studies. In this thesis, we used the approach to test the health effect of 3 servings/day of full-fat dairy products (whole milk, yogurt and cheese) in a swine model of early IR on dyslipidemia and pork quality. In addition, we also compared low-fat vs high-fat dairy products for their effects on cardiometabolic health and immune function in this model. This component of the project was led by a PhD student from our group (Yongbo SHE), and the manuscript can be found in **Appendix B** (published in *Frontiers in Nutrition*).

1.5 Experimental animal models in metabolic research

In general, any non-rodent mammalian animal species used for translational research are defined as large animals such as the swine, dog, sheep, cattle, primates, etc. (Ziegler et al., 2016). Small animals used in research have been foremostly rodents such as rats/mice, rabbits, guinea pigs and hamsters (Russel and Proctor, 2006).

1.5.1 Rodent models used in the field of CVD and T2D

There is no doubt that the rodent has been the most employed animal model in many biomedical fields of research. Rodents have several advantages (over large animals), such as low cost on maintenance, abundant genetic resources and rapid reproduction rates (Ziegler et al., 2016). Although natural rats/mice (not genetically modified nor gene mutated) are typically resistant to develop hyperlipidemia and atherosclerosis. Experimental procedures have been developed to induce these diseases using genetic techniques, dietary intervention (i.e. high fat diet) and/or drugs (i.e. streptozotocin).

C57BL/6 laboratory mouse is one of the most widely used rodent models. The sub-strain C57BL/6J (with single nucleotide polymorphism) is susceptible to diet-induced obesity and obesity-related diabetes (Siersbæk et al., 2020). Another small animal model used by our own research group is the JCR:LA-cp rats (cp stands for corpulent). The homozygous phenotype (cp/cp) of this strain spontaneously develops obesity, IR, hypertriglyceridemia and postprandial dyslipidemia (Russel and Proctor, 2006). **Table 1-2** summarizes some common rodent models used in CVD and T2D research.

Table 1-2. Rodent models used in CVD and T2D research.

Strain or method	Diet	IR	Hyperglycemia	T2D	Obesity	Dyslipidemia	Pathologic islet changes
Genetic mutants/modifications							
Polygenic C57BL/6J mice	HF	√	x	x	√	√	x
Monogenic C57BL/6J–ob/ob mice	Chow, HF	√√	x	x	√√	√	x
Zucker diabetic fatty rat	Chow, HF	√√	√√	x	√√	√	x
NZO mice	CHO	√√	√	√	√√	√	√
JCR:LA-cp rat	HF/CHO	√√	√√	x	√√	√	N/A
Diet-induced							
DIO-sensitive Sprague Dawley rat	HF	√	x	x	√	√	x
DR Sprague Dawley rat	HF	x	x	x	x	x	x
Fructose-induced Wistar rat	CHO	√√	√	x	√	x	N/A
Drug-induced							
Mice or rat injected with Streptozotocin	HF	√	√	√	√	√	√
Other method							
VMH lesion mice and rat	Chow, HF	√	x	x	√	√	x

√√: severe; √: mild: x: absent; Chow: standard diet; HF: high fat diet; CHO: carbohydrate enriched diet; IR: insulin resistance; T2D: type II diabetes; CVD: cardiovascular disease. DIO: diet induced obesity; DR: diet resistance; NZO: New Zealand Obesity strain; VMH: ventromedial hypothalamus; N/A: not applicable. Adapted from Russel and Proctor (2006), Dupas et al. (2016) and Kleinert et al. (2018).

Most of these small animal models have been well-developed and have been used for decades. In contrast, evidence also shows that the mouse has a metabolic rate roughly 7 times that of a human, which can lead to many anatomical, physiological and biochemical variances (Perlman, 2016). For instance, cholesterol metabolism of rodents is predominantly based on HDL (instead of LDL for human and other large animal models). HDL is known to have protective effects against atherosclerosis and other diseases such as diabetes (Kontush, 2014). Therefore, rodent systems and pathophysiology often may not mimic human disease. Large animal models, such as swine, have been long considered in the literature to better translate to the human condition (Vilahur et al., 2011).

1.5.2 Swine as a model for biomedical research

Swine have been proven to be a valuable animal model in nutritional, metabolic and cardiovascular research (Koopmans et al., 2015). They display many similarities to human physiology. With respect to physiology and anatomy, although the pig's intestine length is much longer than humans (15~22m vs 5.5-7m), the ratio of intestine length (m)/body weight (kg) is similar to that for human's (around 0.1 m/kg) (Ziegler et al., 2016). The colon of both the pig and human is sacculated and contains longitudinal muscular bands along their length, which indicates similar transit times, similar intestinal digestive physiology and similar gut microbiomes (Graham 1987; Li et al., 2018). By contrast, the ratio of intestine length/body weight in the mouse and rat is different from that of a human, and the colon is non-sacculated (Graham 1987). This may result in differences in microbial flora within the small intestine and colon, with approximately 85% of mouse microbiome being absent from human flora (Ley et al., 2005). By comparison, both pigs and humans have the similar intestinal microbiome that primarily consists of the Bacteroidetes and Firmicutes phyla (Heinritz et al., 2013). In addition, the pig consumes

an omnivorous diet and has similar food choices and digestive system to humans (Koopsman et al., 2015). This makes the pig particularly suitable to study the effect of diet on metabolism. Yet, the natural progression of T2D seen in humans is difficult to mimic in pigs because diabetes mellitus in pigs is uncommon (Koopsman et al., 2015). Evidence shows that pigs have a large capacity of pancreatic beta-cell population due to strong anabolic and lipogenic ability through long-term domestication (Koopsman et al., 2015). This indicates that pigs are resistant to diabetogenic environment hence typically do not usually develop T2D. Diet-induced diabetes have been observed in Guizhou minipigs, but the elevation of blood glucose level is very limited (Xi et al., 2004). Thus, to use swine model in metabolic research, diabetes is often induced experimentally. One method is through the injection of streptozotocin, which destroys pancreatic beta cells, resulting in development of diabetes (Dufrane et al., 2006). However, drug-induced diabetic animals cannot fully mimic the normal progression of IR to diabetes seen in humans (Wong et al., 2016). Another effective way to induce IR is to utilize low birth weight (LBW) model. Low birth weight swine displays several properties different from normal counterparts in terms of metabolism and growth performance, which will be discussed below.

1.6 Breeding practices in swine production and impact to metabolism

Animals with large litter sizes such as swine can naturally produce the LBW offspring (commonly referred to as “runts”) due to fetal growth restriction (Swanson and David, 2015). The average litter size was increased from 11.9 piglets in 2000 to 14.8 piglets in 2011 (Antonides et al., 2015). Consequently, the average birth weight of piglets has declined about 43g per additional piglet in a litter (Antonides et al., 2015). At the Swine Research Technology Centre (SRTC, affiliated with University of Alberta), the number of piglets born from a sow has been slowly increasing in past decades owing to better breeding technologies, which has led to a

common LBW trend. Birth weight affects the absorption of nutrients due to reduced gut capacity, as well as impaired secretion of digestive enzymes (Wellington et al., 2021). Peptide transporter 1 (PepT1, intestinal oligopeptides transporter), sodium-dependent glucose transporter 1 (SGLT1, intestinal glucose transporter), excitatory amino acid transporter 1 (EAAC1, intestinal glutamate transporter) and sodium-dependent neutral amino acid transporter (B0AT1, intestinal neutral amino acid transporter) have been all reported to be downregulated in the early life stage of LBW piglets (Wellington et al., 2021). Piglets born with LBW exhibit reduced growth rate, fail to the catch up the body weight of their NBW littermates and have a greater risk of preweaning mortality (Fontaine et al., 2019; Feldpausch et al., 2019). This phenomenon gives rise to an economic loss due to reduced postnatal growth potential and efficiency (Wu et al., 2008). The quality of pork from LBW swine is also controversial. Some studies suggest that the pork from LBW pigs has shown unfavorable changes in pH and drip loss, as well as reduced meat tenderness (Gondret et al., 2006; Matyba et al., 2021). While others have reported that birth weight has limited effect on pork quality or carcass traits (Alvarenga et al., 2014; Sundrum et al., 2021). In this study, one of the primary objectives was to evaluate the meat quality and carcass traits of LBW swine as well as the impact of consuming dairy products on these outcomes.

1.7 Low birth weight swine as a model of insulin resistance and dyslipidemia

According to the thrifty gene hypothesis, infants born with LBW (caused by either intra-uterine growth restriction, pre-term birth or poor maternal nutrition) are usually more susceptible to develop metabolic syndrome in early life (Frayling et al., 2001; Li et al., 2017a). Under these conditions, it is thought that nutrient supply is limited and preferentially transported to the brain or lungs to support fundamental development in the fetus. Peripheral tissues can often lack sufficient nutrient supply and result in growth related hormones such as insulin, insulin-like

growth factor (IGF) and insulin-like growth factor binding protein 3 (IGFBP-3) to be downregulated ([1]Li et al., 2017). After birth, when switching from in utero undernutrition to a nutrient-abundant environment, one of the acute compensatory observations is an increased levels of a number of these hormones, resulting in IR within the first several months ([1]Li et al., 2017). In addition, dyslipidemia is another symptom commonly seen in LBW infants (or animals) (Isganaitis et al., 2009; Fontaine et al., 2019; Giretti et al., 2021; Ponzio et al., 2013). A rodent study found an increased lipogenesis in adipose tissue (Genes such as Fasn, Acc1, Insig1, etc. were upregulated) in LBW mice with increased size of adipocytes (Isganaitis et al., 2009). Our own group has previously reported lipid accumulation in muscle and increased intestinal lipid absorption in LBW piglets fed a high fat diet (Fontaine et al., 2019). Circulating plasma TG, VLDL and LDL levels have also been reported to be elevated in either LBW piglets, preterm infants and children born with LBW (Fontaine et al., 2019; Giretti et al., 2021; Ponzio et al., 2013). Consequently, employing a LBW pig model provides an innovative approach to study diet-induced IR, and enables findings to be readily translated to the human condition. Interestingly, despite IR and dyslipidemia being consistently induced in many swine models, differences were observed in HDL-C levels between human patients and swine model of metabolic syndrome (Cluzel et al., 2022). Low HDL-C levels are commonly seen in human patients with metabolic syndrome, while swine models that have been developed tend to exhibit higher HDL-C levels (Cluzel et al., 2022). This is potentially due to the fact that cholesterol is added to the diet to induce hypercholesterolemia in swine, which can increase both LDL-C and HDL-C levels (Cluzel et al., 2022).

One of the current gaps is that the LBW swine model has not been well-studied under different dietary conditions. Currently, there is no common agreement on the definition of LBW

for piglets. Some studies define piglets born < 1kg as low birth weight (Magnabosco et al., 2015; Zheng et al., 2018), while other studies use the range 0.8 to 1.1 kg as LBW (Nebendahl et al., 2013; Nebendahl et al., 2015). To further develop this model, a clear definition between low and normal birth weights may be required and perhaps in a breed-specific manner. In this study, we described the 95% confidence interval (CI) to categorize piglets as LBW (below 95% CI) or NBW (within or above 95% CI).

1.8 Meat Quality

The latter parts of this thesis explored the impact of feeding dairy to LBW piglets on not only the fatty acid composition of pork cuts, but also the overall meat quality from the industry perspective.

Pork is one of the most consumed meats in the world (USDA, 2021). Meat is not only a source of essential nutrients but also possesses specific organoleptic properties that consumers seek such as appearance, taste, texture, etc. Meat quality is defined as factors associated with our sensory perception such as pH, color, juiciness (water-holding capacity, WHC), texture/tenderness, and flavor (Purslow, 2017).

1.8.1 Postmortem Meat pH

pH value was considered as a critical factor affecting meat quality at postmortem (Kim et al., 2016). pH value is typically maintained around 7.0 to 7.2 in the muscle of live pigs (Kim et al., 2016). After slaughter, glycolysis occurs in muscle due to a lack of oxygen supply, causing the accumulation of lactic acid and a decrease in pH (Kim et al., 2016). The postmortem pH "dropping rate" is one the of factors that can determine the meat color and water holding capacity (WHC). Interestingly, WHC further affects drip loss (raw pork) and cooking loss (Kim et al.., 2016). A low pH and a high muscle temperature experienced during early postmortem

causes the breakdown of proteins into peptides or amino acids, resulting in the formation of pale, soft, exudative (PSE) meat (Tomovic et al., 2014). By contrast, if muscle glycogens become depleted too fast during the slaughter process (potentially due to stress, pH at 24-hour postmortem can increase, forming darker, firmer, and dryer (DFD) meat (Tomovic et al., 2014). The DFD meat possesses a higher risk of microbe spoilage (Tomovic et al., 2014). Therefore, both PSE and DFD qualities of meat are considered unfavorable due to the unattractive color, off-flavor taste or greater spoilage risk (Tomovic et al., 2014). The initial pH (at 45-min postmortem) categorizes the pork quality as PSE (pH< 6.0) or normal (pH≥6.0), and the ultimate pH (at 24-hour postmortem) categorizes the pork quality as DFD (pH≥6.0) or normal (pH< 6.0) (**Figure 1-8**) (Tomovic et al., 2014).

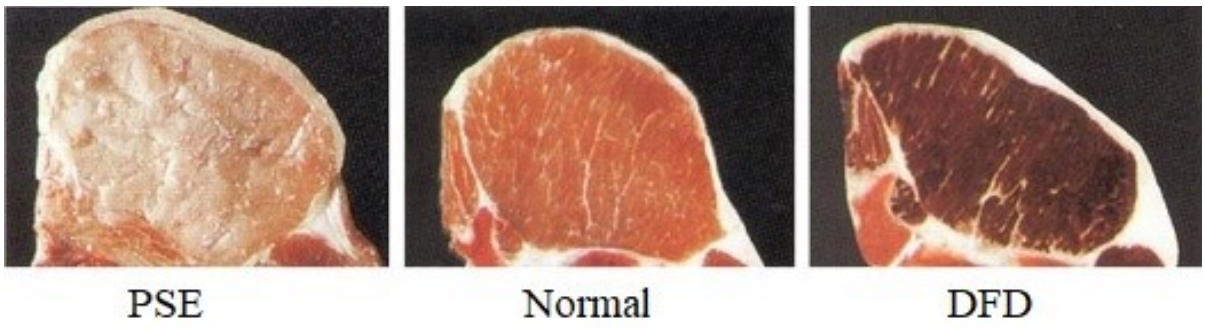

Figure 1-8. Classification of PSE, normal and DFD pork. PSE: pale, soft, exudative; DFD: dark, firm, dry. Adapted from Dawson and Acton, 2018.

1.8.2 Meat Colorization

Myoglobin is the primary factor associated with meat color, which is affected by pH (Suman and Joseph, 2013). It is a sarcoplasmic heme protein that carries and delivers oxygen to tissues with four redox states (Suman and Joseph, 2013). Oxymyoglobin and carboxymyoglobin give a bright cherry-red color (most favorable), while deoxymyoglobin provides a purplish-red

color and metmyoglobin gives an unfavorable brown color (Suman and Joseph, 2013). Metmyoglobin reductase plays a key role in color change during the transformation from muscle to meat (Mikkelsen et al., 1999) and converts metmyoglobin to deoxymyoglobin (Mikkelsen et al., 1999). Ideally, the activity of this enzyme is increased concomitantly with a drop in pH (optimal rate around pH 6.0). However, if pH drop is insufficient, oxymyoglobin will be oxidized to metmyoglobin, causing greater discoloration and the formation of DFD meat (Mikkelsen et al., 1999; Ijaz et al., 2020).

1.8.3 Meat Tenderness

Tenderness is another important factor in determining the meat quality, which can be directly or indirectly affected by a number of factors including: connective tissue (i.e. collagen), pH, aging (proteolysis such as degradation of myofibrillar proteins), muscle type and/or sex (Starkey et al., 2017). Tenderness is measured by shear force or by sensory evaluation (Starkey et al., 2017). Meat with greater tenderness requires less force to palpitate, while tougher meat requires a higher force. One of the proteins determining the myofibrillar structure is desmin, and its degradation can be exacerbated by the enzyme called calpain (Starkey et al., 2017). The degradation of desmin is known to be more rapid at lower pH, which is the reason why PSE meat is usually more tender than normal meat (Starkey et al., 2017). Collagen is the primary protein found in connective tissue, and its concentration is associated with increased meat toughness (Starkey et al., 2017). Both total collagen and collagen solubility can affect shear force (Starkey et al., 2017). Meat collagen can be degraded postmortem by proteolytic enzymes such as metalloproteinase (Weston et al., 2002). The activity of these enzymes is often increased at higher pH (Weston et al., 2002). When heat is applied during cooking, collagen can be transformed to a water-soluble form, increasing the tenderness (Purslow, 2018). Moist heat,

relatively low temperature and slow cooking methods can tenderize the meat to a greater extent (Purslow, 2018). Finally, muscle fibers that are surrounded by the connective tissue can be categorized based on speed on contraction: slow (type I) and fast (type IIA, IIX, and IIB) (Listrat et al., 2016). In pigs, type I, IIA, IIX, and IIB roughly account for 10%, 10%, 25% and 55% of total fibers respectively in *longissimus* muscle, while the composition in *biceps femoris* is typically 15%, 20%, 20% and 45% of total fibers respectively. (Listrat et al., 2016; Kaup et al., 2018). Increased proportions of type I fiber have been found to improve the meat tenderness (Listrat et al., 2016).

1.8.4 Meat Water-Holding Capacity

Type I muscle fiber is also associated with improved juiciness or WHC in meat (Listrat et al., 2016). Water-holding capacity is defined as the ability of the meat to retain its water or added water during cooking or food processing (Listrat et al., 2016). Intramuscular fat is another factor that can influence WHC (Listrat et al., 2016). Meat high in intramuscular fat is considered to have more "marbled" pork. Many Asian cuisines (Chinese, Korean and Japanese) favor marbled pork due to the unique mouthfeel and flavor. Low intramuscular fat level in pork specifically can result in dryer meat (Listrat et al., 2016). By contrast, marbled pork is considered to be juicier, more tender and more palatable than less marbled one according to different sensory evaluations (Brewer et al., 2001; Fernandez et al., 1999). In this thesis, since LBW swine exhibited increased intestinal fat absorption and increased muscle fat deposition (marbled pork), one of the primary objectives was to evaluate the effect of increased dairy intake on meat quality.

To better understand the impact of LBW and dairy intake on intestinal/muscle lipid metabolism and meat quality, we conducted an animal trial utilizing LBW swine model with IR.

The following chapter summarized the current research gap mentioned in chapter 1, as well as presenting rationale, objectives and hypotheses of this thesis.

CHAPTER 2 Rationale, objectives and hypothesis

2.1 Thesis rationale and gap in literature

As we have already mentioned in **Chapter 1**, the prevalence of obesity and obesity related complications such as insulin resistance (IR), type II diabetes (T2D) and cardiovascular disease (CVD) have been increasing dramatically worldwide (World Health Organization, 2021). The thrifty gene hypothesis suggests that low birth weight (LBW) individuals are more susceptible to developing obesity, IR, T2D and dyslipidemia early in life (Frayling et al., 2001; [1]Li et al., 2017). Low birth weight piglets have been shown to exhibit IR and develop preferential intestinal lipid absorption, hypertriglyceridemia and muscle steatosis when fed a high fat (HF) diet compared to normal birth weight (NBW) piglets (Fontaine et al., 2019). Yet, molecular mechanisms associated with intestinal lipid absorption and muscle lipid deposition during these conditions remain unclear. Several fatty acid transporters such as cluster of differentiation 36 (CD36), fatty acid transporter 4 (FATP4) and fatty acid binding protein 2 (FABP2) have been shown to facilitate the uptake of fatty acids in enterocytes and/or myocytes (Dash et al., 2015; Glatz & Luiken., 2017; Said, 2018). Vesicle associated membrane protein 7 (VAMP7) has also been found to play a functional role on the regulation of intestinal pre-chylomicron transport vesicle (PCTV) together with CD36 (Siddiqi et al., 2010). Additionally, LBW swine are not often valued in the industry and/or on the market due to reduced growth rate and meat quality, although these negative impacts are controversial in the literature. We have identified that preferential lipid deposition in muscle tissue may yield a human health benefit by increasing the proportion of beneficial fatty acids for meat consumption. In this regard, this project may help add value to LBW swine in order to compensate for some of the economic loss.

Dairy-derived fatty acids such as myristic acid (C14:0), pentadecanoic acid (C15:0) and margaric acid (C17:0) have been shown to provide beneficial effects on insulin sensitivity, which can be potentially incorporated in pork muscle (Zhuang et al., 2019; Imamura et al., 2018; Amine et al., 2021). Dairy ingredients (such as lactose and whey protein) have also been shown to increase growth in weaning piglets (Yun et al., 2005; Cromwell et al., 2008).

2.2 Objectives and hypotheses

The overall objective of this thesis was to explore how LBW and dairy intake may modulate intestinal lipid absorption and lipid incorporation in muscle, as well as how they impact the overall meat quality. Three objectives and corresponding hypotheses were proposed:

1. To verify the LBW swine model to develop an IR phenotype. We hypothesized that LBW swine would develop IR, hypertriglyceridemia and muscle steatosis when fed a HF diet, as observed in a previous study using the same approach (Fontaine et al., 2019).
2. To investigate intestinal/muscle lipid metabolism in LBW swine. Two specific sub-objectives were proposed:
    a. To delineate the potential molecular transport candidates responsible for intestinal lipid absorption and muscle lipid deposition in LBW swine. We considered that the protein expression of candidate transporters (CD36, FABP2, FATP4 and VAMP7) in enterocytes and/or myocytes in LBW swine could potentially explain the development of hypertriglyceridemia and ectopic lipotoxicity.
    b. To determine dietary fatty acid incorporation in pork while consuming increased dairy. We proposed that increasing dietary intake of dairy-derived fatty acids would result in a higher proportion of these fatty acids being incorporated into the muscle, likely facilitated by candidate fatty acid transporters.

3. To elucidate the impact of LBW and increased dairy intake on growth performance, carcass traits and meat quality, as well as muscle fibers and collagen contents in *longissimus* muscle. We hypothesized that LBW swine would exhibit unfavorable changes on postnatal growth, pork quality or carcass characteristics compared to NBW counterparts. We also proposed that consuming 3 servings/day of full-fat dairy products might compensate for growth impairment in LBW swine, possibly improving pork quality.

2.3 Chapter format

Chapter 3 "Preferential deposition of dairy derived fatty acids in muscle tissue is partially due to the upregulation of CD36 in a low-birth-weight swine model" investigates the impact of dairy on IR, the mechanistic pathway of intestinal and muscle lipid metabolism in LBW swine, and dietary fatty acids accumulation in pork with increased dairy intake**.** Objectives 1 and 2 have been addressed in Chapter 3. Findings from Chapter 3 show that LBW swine fed a HF diet developed early signs of IR and can be ameliorated by increased dairy intake. LBW-HF swine also exhibited increased triglyceride content in muscle compared to NBW-Chow group. CD36 was upregulated in both small intestine and muscle in LBW swine, potentially explaining the increased intestinal lipid absorption and preferential muscle lipid deposition in LBW swine. We also found that the consumption of dairy can enhance the incorporation of long-chain PUFA into LBW pork.

Personal contributions: I took a lead role in this study and was involved in daily animal care, measuring protein expression and fatty acid composition with the assistance of students and staff from the Proctor, Richard and Bruce labs and the animal facility. Chapter 3 is an original

scientific study and one of the main studies contributing to this thesis. The manuscript has been submitted to *Frontiers in Veterinary Science* in July 2022 (currently under review).

Chapter 4 "**Low birth weight swine did not exhibit major defects on growth performance, meat quality or carcass traits**" compares growth performance, meat quality and carcass traits between NBW swine vs LBW swine. Objective 3 was addressed in this chapter. Findings from Chapter 4 illustrate that no significant differences were found in growth performance, overall meat and carcass quality between these two groups in the context of a HF diet. Dairy products may play an important role in postnatal myogenesis and increasing the carcass lean yield in LBW swine.

Personal contributions: I took a lead role in this part of the study on daily animal care, helping with surgery and tissue collections with the assistance of students and staff from the Proctor and Richard labs and the animal facility. I also assisted Dr. Bimol Roy and students from the Bruce lab in conducting experiments related to meat/carcass quality. Chapter 4 is an adaptation of a research paper led by Dr. Bimol Roy (**Appendix C**). The manuscript is in preparation and will be submitted to *Canadian Journal of Animal Science*.

Chapter 5 provides a general overall discussion including results from each study, their implications, as well as considerations for future direction.

2.4 Ethical considerations and procedure developments

A major part of this thesis was dedicated to developing the LBW swine model with early IR in nutritional research. Piglets were obtained from the bio-secure Swine Research and

Technology Center (SRTC), Department of Agriculture, Food and Nutritional Science, University of Alberta, Canada. All procedures were in accordance with the Canada Council on Animal Care (CCAC) guidelines and approved by the University of Alberta's Animal Ethics Committee. Piglets were the product of a cross between a Duroc boar and Large White/Landrace sows. All living conditions (water, food, temperature, hygiene, etc.) were provided properly by trained staff under the guidelines of animal ethics. Our laboratory and the staff from SRTC have invested significant time to ensure each piglet in this thesis was taken care of with a high quality of life. We made sure each piglet was socialized on a daily basis to minimize stress response during subsequent experimental procedures. Piglets that did not successfully adapt to the research environment such as exhibiting aggression, anxiety or anorexia were excluded from the study in order to alleviate the distress. Animal surgery was handled by senior veterinarians and skilled personnel to ensure high standards of animal welfare. Additionally, facility staff took daily records to ensure piglets were healthy without adverse reactions.

CHAPTER 3: Preferential deposition of dairy derived fatty acids in muscle tissue is partially due to the upregulation of CD36 in a low-birth-weight swine model

The manuscript of this Chapter has been submitted to *Frontiers in Veterinary Science* (in review)

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3.1 Background

Metabolic syndrome (MetS) is a worldwide health issue with the prevalence rising dramatically in the past decade (Saklayen, 2018). Large animal models (such as swine) are now being developed to routinely study metabolic diseases in order to better translate to the human condition (Zhang and Lerman, 2016). Our previous research has established an innovative approach to study MetS and insulin resistance (IR) in a low birth weight (LBW) swine model. Results revealed that LBW swine fed a high fat (HF) diet were susceptible to IR and developed a preferential intestinal lipid absorption, hypertriglyceridemia and muscle steatosis (Fontaine et al., 2019). It is well known that in many animal models, a HF diet can lead to increased lipid absorption and over-production of chylomicrons, which further contributes to hypertriglyceridemia. Hypertriglyceridemia has been associated with ectopic lipid accumulation in peripheral tissues such as the liver and muscle (Zou et al., 2020). Yet, the molecular understanding of the mechanisms associated with intestinal lipid absorption and muscle lipid deposition during IR remain unclear. Long-standing evidence suggests that fatty acid transporters

such as cluster of differentiation 36 (CD36), fatty acid transporter 4 (FATP4) and fatty acid binding protein 2 (FABP2) are expressed on the surface membrane of enterocytes and/or myocytes and can facilitate the uptake of long chain fatty acids into tissues (Lewis et al., 2015; Glatz & Luiken. 2017; Said, 2018). Vesicle associated membrane protein 7 (VAMP7) was also found to play a functional role on the regulation of intestinal pre-chylomicron transport vesicle (PCTV) (Siddiqi et al., 2010). In this study, we hypothesized that protein expressions of these candidate transporters in enterocytes and/or myocytes in LBW swine could potentially explain the development of hypertriglyceridemia and ectopic lipotoxicity, which implicate post-translational regulation.

Low birth weight swine are often not valued in the pork production industry due to reduced growth rate and limited economic gain. However, we also have identified that preferential lipid deposition in muscle tissue may yield a human health benefit by increasing the proportion of beneficial fatty acids. Dairy-derived fatty acids such as myristic acid (C14:0), pentadecanoic acid (C15:0) and margaric acid (C17:0) have been shown to be valid biomarkers to assess dairy intake, which can be utilized to investigate the specific muscle lipid incorporation (Biong et al., 2006). Additionally, there has been significant debate over the impact of dairy consumption on both cardiometabolic health and glycemic control in humans. Interestingly, odd chain fatty acids (C15:0 and C17:0) from dairy have been associated with lower risk of type II diabetes and total mortality (Zhuang et al., 2019; Imamura et al., 2018). As a result, we have proposed that increasing dairy product intake in LBW swine may be a suitable approach to alleviate metabolic consequences of IR specifically in LBW swine.

The overall aim of this study was to delineate the molecular transport candidates responsible for intestinal lipid absorption and muscle lipid deposition in LBW swine; and secondly to determine

dietary fatty acids accumulation in pork with increased dairy intake. We hypothesized that the consumption of 3 servings/day of full-fat dairy products would improve insulin sensitivity. We also proposed that increasing dietary intake of dairy-derived fatty acids would result in higher proportion of these fatty acids being incorporated into the muscle, likely facilitated by candidate fatty acid transporters.

3.2 Study designs and Methodology

3.2.1 Animal and housing:

Details regarding animal care and ethics were described in She et al. (2022). Briefly, pigs were obtained from the bio-secure Swine Research and Technology Center (SRTC), Department of Agriculture, Food and Nutritional Science, University of Alberta, Canada. All procedures were in accordance with the Canada Council on Animal Care (CCAC) guidelines and approved by the University of Alberta's Animal Ethics Committee. Piglets were the product of a cross between a Duroc boar and Large White/Landrace sows. All living conditions (water, food, temperature, hygiene, etc.) were provided properly by trained staff under the guidelines of animal ethics.

3.2.2 Study design and diets:

Details regarding study design and experimental diets were described in She et al. (2022). At birth, full-term newborn male piglets were weighed and selected based on normal birth weight (NBW) and low birth weight (LBW). A 95% confidence interval (CI) was used to categorize piglets as LBW (below 95% CI) or NBW (within or above 95% CI). All piglets were fed a standard grower diet after weaning until 5-week of age. At 5-week of age, NBW and LBW piglets were fed a 1-week transition diet, then were randomly assigned to three experimental diets: 1-chow diet, 2-HF diet or 3-HF diet supplemented with 3 servings per 2000kcal/day of full fat dairy products. The groups are as follow: NBW-Chow (n=5), NBW-HF (n=6), LBW-HF

(n=8) and LBW-HF+Dairy (n=5). Chow and HF diet were provided ad libitum. Both HF and HF +Dairy diets were isocaloric, and piglets on HF+Dairy diet were pair-fed to piglets on HF diet to ensure similar energy intake. The nutritional composition of chow and HF diets has been previously described (Fontaine et al., 2019). In short, HF diet consisted of 46%, 33% and 21% of total energies from fat (mainly lard), carbohydrates (mainly fructose) and protein respectively. 1% w/w cholesterol was added. Fishmeal was added as a source of protein and long chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (**Table 3-1**). On the contrary, chow diet consisted of 14%, 69% and 17% of total energies from fat, carbohydrates and protein respectively. Dairy extract (high-lactose whey) was added to support the growth of piglets. Regarding HF+Dairy diet, servings of dairy products (per 2000 kcal/day) included: whole milk powder (3.25% fat, 33g; Bulk Barn, Canada), plain yogurt (10% fat, 175ml; Liberté, Canada) and mozzarella cheese (28% fat, 50g; No Name®, Canada). As shown in **Table 3-1**, the chow diet contained more alpha linoleic (C18:2n6) and alpha linolenic (C18:3n3) acids and less oleic (C18:1n9) and saturated fatty acids (palmitic & steric acids) in general than the HF diet. Incorporating dairy as part of the HF diet increased the proportion of medium chain fatty acids (i.e. C10:0, C12:0, C14:0 and C14:1) along with dairy specific fatty acids (i.e. C15:0, C17:0 and C18:1t11) in the diet. This was also associated with lower proportion of C18:1n9, C18:2n6, EPA and DHA when incorporating dairy in the HF diet (**Table 3-1**).

Table 3-1. Fatty acids compositions of different experimental diets (% of total fatty acids).

Fatty acids	**Chow**	**HF**	**HF+Dairy**
C10:0	0.06	0.02	0.59
C12:0	<0.01	<0.01	1.24

C14:0	0.14	1.60	5.72
C14:1	<0.01	0.01	0.40
C15:0	0.85	0.18	0.62
C16:0	17.93	25.48	29.82
C16:1n9	<0.01	0.28	0.25
C16:1n7	0.19	2.12	2.21
C17:0	0.15	0.32	0.50
C18:0	2.45	13.69	12.87
C18:1t11	<0.01	0.23	0.65
C18:1n9	24.21	33.67	29.52
C18:1n7	1.58	2.94	2.06
C18:2n6	48.15	15.92	10.86
C18:3n6	<0.01	0.03	0.03
C18:3n3	3.49	1.44	1.06
C20:0	0.31	0.22	0.19
C20:1n9	0.48	0.59	0.41
C20:2n6	<0.01	0.50	0.33
C20:3n3	<0.01	0.05	0.07
C20:4n6	<0.01	0.14	0.13
C20:5n3 (EPA)	<0.01	0.32	0.22
C22:5n3 (DPA)	<0.01	<0.01	0.07
C22:6n3 (DHA)	<0.01	0.27	0.17
Total SFA	21.91	41.53	51.56
Total MUFA	26.49	39.82	35.50
Total PUFA	51.71	18.68	12.94

Total n-6	48.19	16.63	11.42
Total n-3	3.52	2.03	1.52
n-6/n-3 ratio	13.70	8.17	7.49

Data are presented as a % of total fatty acids. HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n-6: omega 6 fatty acids: n-3: omega 3 fatty acids.

Food intake and body weight were measured on a daily or weekly basis respectively. At 12-week of age, piglets were fasted overnight, and fasting blood was collected using capillary tubes. Piglets were then euthanized, bled and eviscerated by trained staff at SRTC, followed by tissue collection (**Figure 3-1.**).

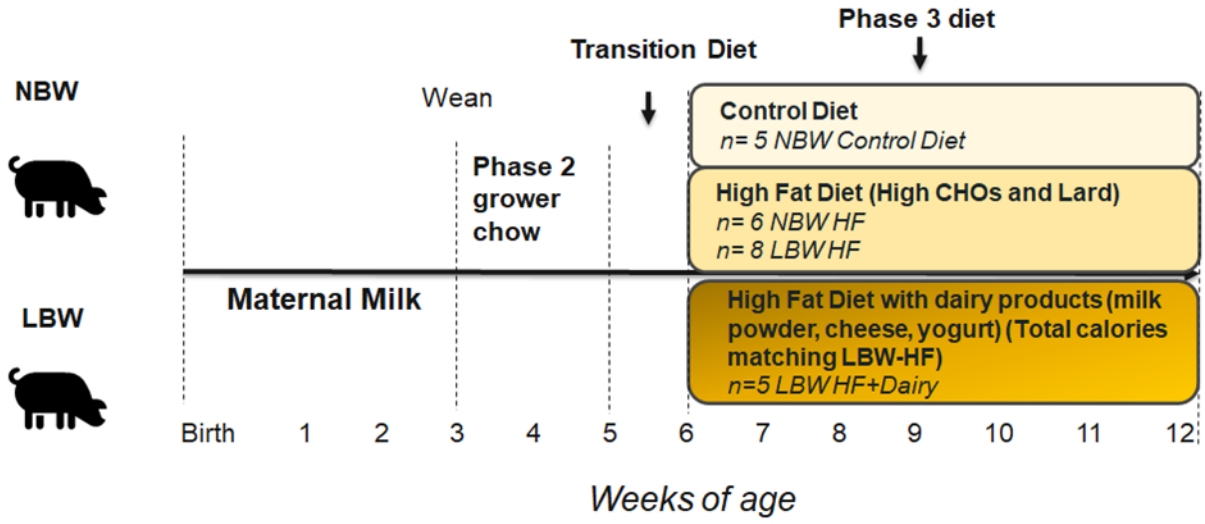


Figure 3-1. Study design to investigate intestinal and muscle lipid metabolism under the impact of dairy products in LBW swine model of insulin resistance. CHO: carbohydrate; NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

3.2.3 Sample collection and processing:

Fasting blood samples along with muscle and mucosal scrapings (jejunum) were collected and flushed with ice-cold sterile PBS (phosphate buffered saline, pH 7.4). Tissue samples were snap frozen in liquid nitrogen before being stored in a -80°C freezer.

3.2.4 Biochemical analysis:

Fasting plasma samples were assessed for glucose concentrations and lipid profiles using commercially available colorimetric kits for glucose, triglycerides (TG), total cholesterol (TC),

low-density lipoprotein (LDL) and high-density lipoprotein (HDL). All kits were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Fasting insulin levels were measured via porcine-specific enzyme-linked immunosorbent assay (ELISA) kits (Mercodia, USA). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was used to evaluate IR (Matthews et al.,1985).

Triglyceride in jejunum mucosal scrapings and muscle samples (*biceps femoris*) was determined by the colorimetric test (Wako Pure Chemical Industries Ltd, Tokyo, Japan). Samples were pulverized using liquid nitrogen in radio-immuno-precipitation assay (RIPA) buffer and centrifuged at 4800 rpm for 10 mins, and supernatant was collected. Triglyceride concentration of the supernatant was measured using the same colorimetric kit as mentioned above.

3.2.5 Fatty acids composition:

Diet sample, plasma and ham muscle (*biceps femoris*) were used to assess fatty acid compositions via gas chromatography equipped with a flame ionization detector (Agilent 8890) as described previously (She et al., 2022). Briefly, diet and muscle samples were homogenized and only homogenates were collected. Total lipids were extracted by the Folch method, saponified and then methylated with hexane and BF3 (boron trifluoride) as described previously (Folch et al., 1957; Cruz-Hernandez et al., 2004).

Fatty acid methyl esters were identified by chromatography with GLC standards (Sigma-Aldrich) and from the NIST 05 MS Library Database. Quantification of the fatty acid methyl ester profiles was made by considering the relative peak areas, expressed as the relative percentage of the individual area of each one as related to the total area of compounds in the chromatogram.

3.2.6 Protein analysis:

Western blot was used to determine candidate fatty acid transporters expression in mucosal scrapings of the jejunum and ham muscle (*biceps femoris*), adapted from Huang et al., (2022) and Krysa et al., (2020).

Briefly, after homogenization, proteins were separated by SDS-PAGE on a 4~12% Bis-tris NuPAGE Gel (ThermoFisher Scientific, Waltham, MA, USA) using the BioRad Criterion System and electro-blotted to polyvinylidene difluoride (PVDF) membranes. Membranes were incubated overnight with primary antibodies (Anti-CD36, #ab133625, Abcam; Anti-FABP2, #ab231070, Abcam; Anti-FATP4, #ab200353, Abcam; Anti-VAMP7, #NBP2-41183, Novus Biologicals; Anti-beta actin, #ab8226, Abcam), followed by the incubation of corresponding secondary antibodies. Protein bands were visualized by ECL luminescent solution. ImageJ (https://imagej.nih.gov/ij/, NIH, USA) was used for the quantification of selected proteins.

3.2.7 Carcass traits

Swine carcasses were transported to Agri-Food Discovery Place (University of Alberta, Edmonton, Alberta, Canada) immediately and weighed (recorded as hot carcass weight) before being stored in a 4°C cooler for 24 hours.

Subcutaneous fat depth, loin muscle depth and subjective marbling score were all measured by using a steak (2.54cm) between the last 3rd and 4th rib of loin eye muscle. The maximum depth of the muscle perpendicular to the maximum of the width was measured to determine loin muscle depth, and subcutaneous fat depth was measured in the same line of the muscle depth according to Teixeira et al. (2021). Subjective marbling score was assessed by trained personnel based on National Pork Board Pork Quality Standards on a scale from 0 to 10 where 0=devoid and 10=abundant presence of marbling. Measurements of subcutaneous fat depth and muscle

depth were also used to calculate the predicted leanness, namely Canadian Lean Yield %, using the formula from Pomar and Marcoux (2003).

The proximate analysis to determine the crude fat content (intramuscular fat) has been described previously (Roy et al., 2021; Roy et al., 2018). Briefly, after muscle and fat depth measurements, epimysium and external fat in the steak were trimmed off, cut into small cubes, frozen, lyophilized and grounded, followed by crude fat extraction.

3.2.8 Statistical analysis:

Data are reported as mean±SEM. IBM SPSS (version 26.0) was used for statistical analysis. Fasting data were analyzed by one-way ANOVA with diet as the main effect and in cases where a p-model was significant, post-hoc comparison tests was performed with the Tukey's HSD adjustment for multiple comparisons among groups. For fatty acids profile, since dairy extract was added into the chow diet to support the piglets' growth, the LBW-HF control group was used for comparison with the LBW-HF+Dairy and was compared directly by student's t-test. All tests with p-value <0.05 were considered statistically significant.

3.3 Results

3.3.1 Growth performance and food intake

As expected, all LBW piglets had significantly lower birth weight than NBW piglets ($p<0.05$, **Table 3-2**). Chow-fed NBW piglets had significantly lower average daily energy intake and energy to gain ratio, compared with HF-fed piglets ($p<0.05$, **Table 3-2)**. No other significant differences were found in terms of average daily gain and final body weight among all groups.

Table 3-2. Growth performance and feed consumption of NBW and LBW swine fed experimental diets.

Growth and feed intake	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-model*
Birth weight, kg	1.620 ± 0.066[a]	1.733 ± 0.095[a]	1.228 ± 0.081[b]	1.225 ± 0.065[b]	<0.001
Final weight, kg	48.44 ± 3.164	53.62 ± 1.970	48.10 ± 2.771	45.64 ± 1.22	0.10
Average daily gain, g/d	779.2 ± 47.34	854.0 ± 15.80	794.7 ± 41.00	753.2±49.4	0.30
Average daily energy intake, kcal/d	4076.9 ± 290[a]	5716.7 ± 340[b]	5313.5 ± 238[b]	5239.9±52.3[b]	<0.01
Energy to gain ratio	5.23 ± 0.14[a]	6.67 ± 0.29[b]	6.72 ± 0.15[b]	6.98±0.22[b]	<0.01

Values are shown as means ±SE. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products. This table is also presented in She et al. (2022).

3.3.2 Fasting plasma biomedical variables in response to HF diet and/or dairy product.

High fat-fed LBW swine exhibited significantly higher fasting plasma glucose level ($p<0.05$) and tended to have higher fasting plasma insulin ($p=0.091$) and HOMA-IR score ($p=0.086$) than Chow-fed NBW swine (**Table 3-3**). Feeding dairy products as a part of a HF diet in LBW swine improved plasma glucose concentration to values no longer significantly different from NBW-Chow (**Table 3-3**). High fat diet significantly elevated fasting TC and LDL levels compared with the chow diet, regardless of dairy intake (**Table 3-3**).

Table 3-3. Fasting plasma biochemistry in NBW and LBW swine fed experimental diets at 12-week of age.

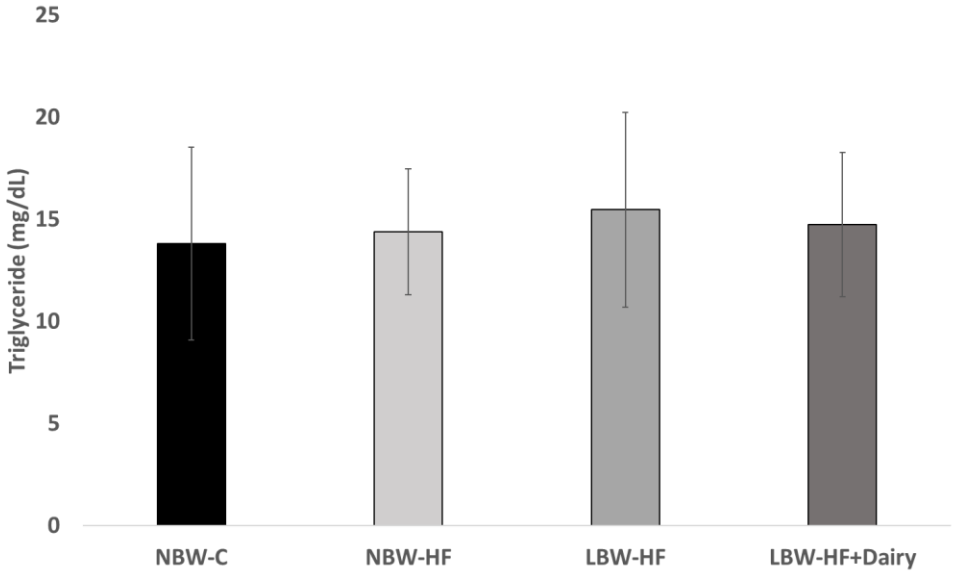
Fasting plasma levels	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-value*
Glucose, mmol/L	4.44 ± 0.31^{a}	5.99 ± 0.29^{b}	6.27 ± 0.36^{b}	5.18 ± 0.28^{ab}	0.004
Insulin, mU/L	3.66 ± 0.36	5.63 ± 0.88	9.91 ± 3.27	3.59 ± 0.58	0.091
HOMA-IR	0.72 ± 0.07	1.52 ± 0.27	3.04 ± 1.19	0.83 ± 0.15	0.086
TC, mg/dL	92.3 ± 4.4^{a}	300.2 ± 25.4^{b}	252.6 ± 17.4^{b}	241.1 ± 24.0^{b}	<0.001
LDL, mg/dL	54.1 ± 2.0^{a}	177.4 ± 14.5^{b}	158.3 ± 15.4^{b}	145.2 ± 13.7^{b}	<0.001
HDL, mg/dL	24.4 ± 3.1	26.1 ± 3.5	24.1 ± 2.8	19.5 ± 1.0	0.469
TG, mg/dL	24.3 ± 2.6	25.2 ± 4.9	29.4 ± 2.1	38.7 ± 10.5	0.308

Values are presented as mean±SE. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglyceride.

3.3.3 TG contents in ham muscle and mucosal scrapings of jejunum

Despite no difference in circulating concentrations of TG, muscle samples from HF-fed LBW swine contained significantly higher TG content compared to Chow-fed NBW swine ($p<0.05$, **Figure 3-2B**). The introduction of a dairy source for dietary fat did not change muscle TG content in LBW-HF+Dairy group (**Figure 3-2B**). No significant difference was observed in intestinal TG content among groups (**Figure 3-2A**).

A. TG concentration in mucosal scrapings



B. TG concentration in muscle

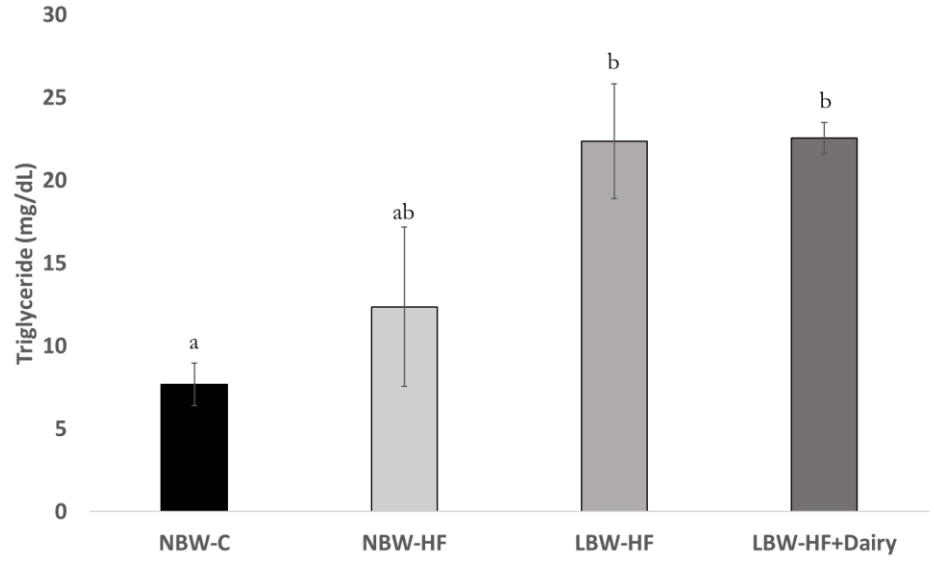


Figure 3-2. Comparison of TG levels (mg/dl) in mucosal scrapings (**A**) and ham muscle (biceps femoris) (**B**) in NBW and LBW swine fed experimental diets at 12-week of age. Values are presented as mean±SEM. TG: triglyceride; NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

3.3.4 Fatty acid profile in plasma

High fat feeding significantly increased plasma levels of eicosapentaenoic acid (EPA, C20:5n3) in NBW and LBW swine compared with Chow-fed NBW swine ($p<0.05$, **Table 3-4)**. Further, feeding dairy products significantly increased margaric acid (C17:0) possibly at the expense of EPA (C20:5n3) when compared to LBW swine fed a HF diet ($p<0.05$, **Table 3-4**).

Table 3-4. Fatty acids compositions in plasma (%) in NBW and LBW swine fed experimental diets at 12-week of age.

Fatty acids	**NBW-Chow**	**NBW-HF**	**LBW-HF**	**LBW-HF+Dairy**	***P-value*[1]**	
					1	**2**
C14:0	1.5±0.09[a]	1.23±0.06[ab]	1.04±0.07[b]	1.02±0.13[b]	0.003	0.861
C15:0	0.7±0.05[a]	0.53±0.04[b]	0.44±0.03[b]	0.43±0.03[b]	<0.001	0.918
C16:0	22.11±1.27[ab]	20.12±0.24[a]	21.55±0.69[ab]	23.99±0.92[b]	0.032	0.055
C17:0	0.98±0.11[a]	0.47±0.02[b]	0.5±0.03[b]	0.68±0.09[b]	<0.001	0.043
C18:0	18.99±1.06	18.23±0.49	19.49±0.76	21.34±1.15	0.132	0.188
C18:1	30.12±1.30[a]	30.86±0.49[a]	29.44±0.63[a]	26.31±0.69[b]	0.005	0.008
C18:2n6	17.86±0.24	20.21±0.60	19.36±0.69	18.34±0.42	0.058	0.301
C18:3n6	0.14±0.03	0.16±0.01	0.16±0.03	0.2±0.03	0.640	0.469
C18:3n3	0.24±0.03	0.16±0.02	0.2±0.05	0.17±0.02	0.469	0.680
C20:0	1.15±0.16[a]	0.71±0.03[b]	0.65±0.04[b]	0.57±0.03[b]	<0.001	0.233
C20:1n9	0.21±0.02	0.19±0.01	0.19±0.01	0.16±0.01	0.27	0.203

C20:2n6	0.32±0.01[ab]	0.32±0.01[a]	0.29±0.01[ab]	0.26±0.03[b]	0.029	0.272
C20:3n3	0.32±0.04[a]	0.65±0.02[b]	0.65±0.05[b]	0.76±0.10[b]	<0.001	0.318
C20:4n6	3.47±0.58	2.86±0.17	2.8±0.20	2.87±0.28	0.442	0.830
C20:5n3 (EPA)	0.34±0.06[a]	0.96±0.05[b]	0.96±0.10[b]	0.65±0.05[a]	<0.001	0.04
C22:5n3 (DPA)	0.37±0.08	0.34±0.03	0.31±0.03	0.29±0.04	0.691	0.681
C22:6n3 (DHA)	0.25±0.06[a]	0.91±0.07[b]	0.85±0.10[b]	0.83±0.13[b]	<0.001	0.932
Others	0.94±0.11	1.10±0.06	1.11±0.05	1.11±0.06	0.247	0.936
Total SFA	45.43±1.94[ab]	41.28±0.63[a]	43.67±1.11[ab]	48.04±0.89[b]	0.008	0.019
Total MUFA	31.26±1.41[a]	32.15±0.52[a]	30.74±0.68[ab]	27.58±0.75[b]	0.01	0.012*
Total PUFA	23.31±0.97	26.57±0.60	25.59±0.84	24.38±0.60	0.059	0.327
Total n-6	21.79±0.81	23.56±0.55	22.61±0.69	21.67±0.42	0.216	0.343
Total n-3	1.52±0.16[a]	3.01±0.12[b]	2.97±0.27[b]	2.71±0.28[b]	0.001	0.526
n-6/n-3 ratio	14.79±1.06[a]	7.87±0.30[b]	8.03±0.70[b]	8.35±0.86[b]	<0.001	0.780

Values are presented as means±SE. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n-6: omega 6 fatty acids: n-3: omega 3 fatty acids. Others: all remaining fatty acids that were too low to be quantified and/or could not be identified with current standards used. [1]Contrast: 1=one way ANOVA; 2=LBW-HF vs LBW-HF+Dairy.

3.3.5 Fatty acid profile in ham muscle

High fat feeding significantly reduced the n-6/n-3 ratio in *biceps femoris* from both NBW and LBW swine compared with Chow-fed NBW swine ($p<0.05$, **Table 3-5**). Feeding dairy products increased myristic acid (C14:0), docosapentaenoic acid (DPA, C22:5n3) and docosahexaenoic acid (DHA, C22:6n3) relative to high fat feeding alone ($p<0.05$, **Table 3-5**).

Table 3-5. Fatty acids compositions in ham muscle (biceps femoris) (%) in NBW and LBW swine fed experimental diets at 12-week of age.

Fatty acids	**NBW-Chow**	**NBW-HF**	**LBW-HF**	**LBW-HF+Dairy**	***P-value*[1]**	
					1	**2**
C14:0	1.16±0.05[a]	1.27±0.03[a]	1.25±0.05[a]	2.28±0.20[b]	<0.001	<0.001
C15:0	0.38±0.26	0.17±0.04	0.26±0.09	0.28±0.03	0.765	0.319
C16:0	23.79±1.27	23.15±0.33	23.23±0.50	25.03±0.29	0.301	0.027
C17:0	0.44±0.18	0.22±0.05	0.29±0.02	0.38±0.04	0.520	0.12
C18:0	12.23±0.71	13.18±1.38	13.73±1.44	13.45±0.51	0.839	0.884
C18:1	38.63±3.04	40.93±1.37	39.52±1.15	37.03±0.83	0.528	0.153
C18:2n6	16.04±0.95	13.85±0.68	14.68±0.88	13.65±0.99	0.260	0.468
C18:3n6	0.05±0.02	0.02±0.006	0.03±0.01	0.02±0.01	0.474	0.583
C18:3n3	0.14±0.02	0.16±0.01	0.14±0.02	0.1±0.02	0.247	0.204
C20:0	1.55±0.25[a]	0.95±0.06[ab]	0.99±0.14[ab]	0.73±0.06[b]	0.014	0.208

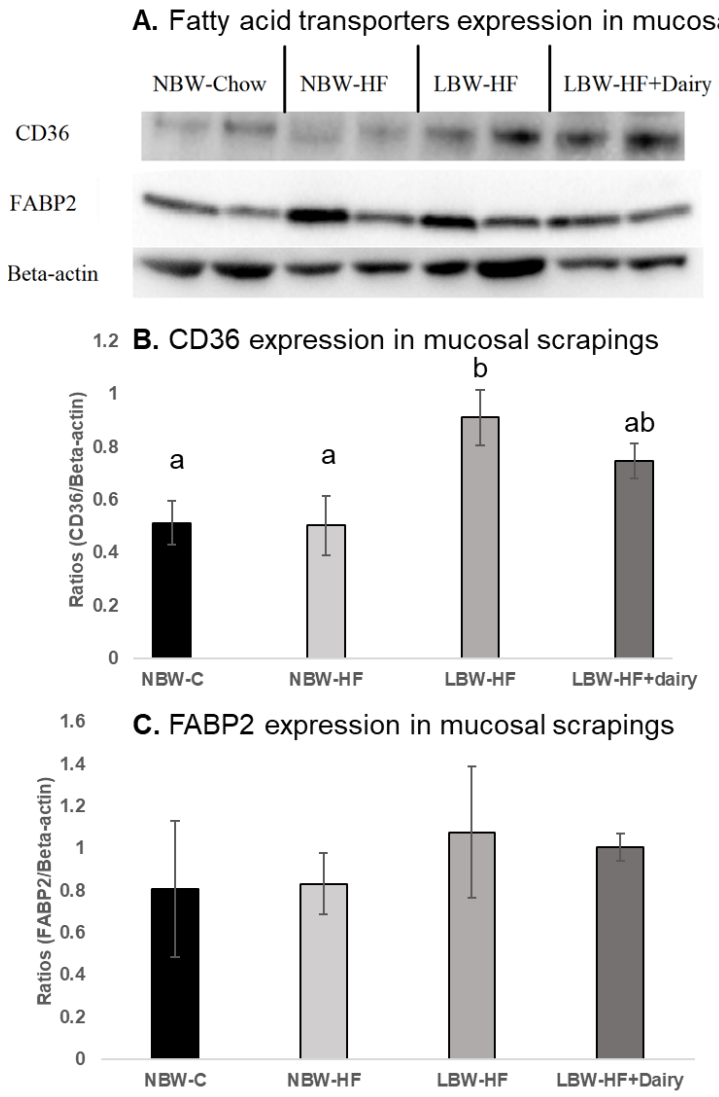
C20:1n9	0.53±0.10	0.59±0.03	0.55±0.04	0.46±0.03	0.420	0.121
C20:2n6	0.45±0.07	0.52±0.02	0.5±0.03	0.44±0.02	0.505	0.191
C20:3n3	0.22±0.04[a]	0.27±0.04[ab]	0.3±0.04[ab]	0.39±0.04[b]	0.082	0.209
C20:4n6	1.43±0.29	1.11±0.26	1.28±0.21	1.79±0.34	0.406	0.207
C20:5n3 (EPA)	0.26±0.11	0.38±0.06	0.38±0.06	0.48±0.08	0.357	0.392
C22:5n3 (DPA)	0.28±0.10	0.32±0.04	0.3±0.02	0.43±0.05	0.366	0.038
C22:6n3 (DHA)	0.24±0.16	0.44±0.03	0.4±0.08	0.54±0.05	0.110	0.092
Others	2.19±0.22	2.45±0.26	2.11±0.20	2.41±0.22	0.648	0.345
Total SFA	39.55±2.12	38.95±1.31	39.79±1.49	42.15±0.61	0.565	0.255
Total MUFA	41.34±3.29	43.96±1.59	42.19±1.33	40.0±1.09	0.593	0.278
Total PUFA	19.10±1.30	17.07±1.09	18.02±1.10	17.85±1.52	0.698	0.929
Total n-6	17.97±1.19	15.50±0.94	16.49±1.07	15.91±1.32	0.448	0.739
Total n-3	1.14±0.40[a]	1.56±0.15[ab]	1.52±0.17[ab]	1.94±0.21[b]	0.02	0.076
n-6/n-3 ratio	21.12±4.13[a]	10.08±0.42[b]	11.78±2.01[b]	8.27±0.28[b]	0.006	0.100

Values are presented as means±SE. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n-6: omega 6 fatty acids: n-3:

omega 3 fatty acids. Others: all remaining fatty acids that were too low to be quantified and/or could not be identified with current standards used. [1]Contrast: 1=one way ANOVA; 2=LBW-HF vs LBW-HF+Dairy.

3.3.6 Expression of candidate fatty acid transporters

All high fat-fed LBW swine (regardless of dairy intake) exhibited an upregulation of CD36 expression compared with NBW littermates in both small intestine (**Figure 3-3A, B**) and muscle (*biceps femoris*) (**Figure 3-3D, E**) ($p<0.05$). No significant difference was found regarding the expression of FABP2 among groups (**Figure 3-3A, C**). No significant differences were found regarding the expression of other candidate transporters or related proteins such as FATP4 and VAMP7 (data not shown).


A. Fatty acid transporters expression in mucosal scrapings
NBW-Chow
NBW-HF
LBW-HF
LBW-HF+Dairy
CD36
FABP2
Beta-actin
B. CD36 expression in mucosal scrapings
Ratios (CD36/Beta-actin)
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
ab
NBW-C
NBW-HF
LBW-HF
LBW-HF+dairy
C. FABP2 expression in mucosal scrapings
Ratios (FABP2/Beta-actin)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NBW-C
NBW-HF
LBW-HF
LBW-HF+Dairy

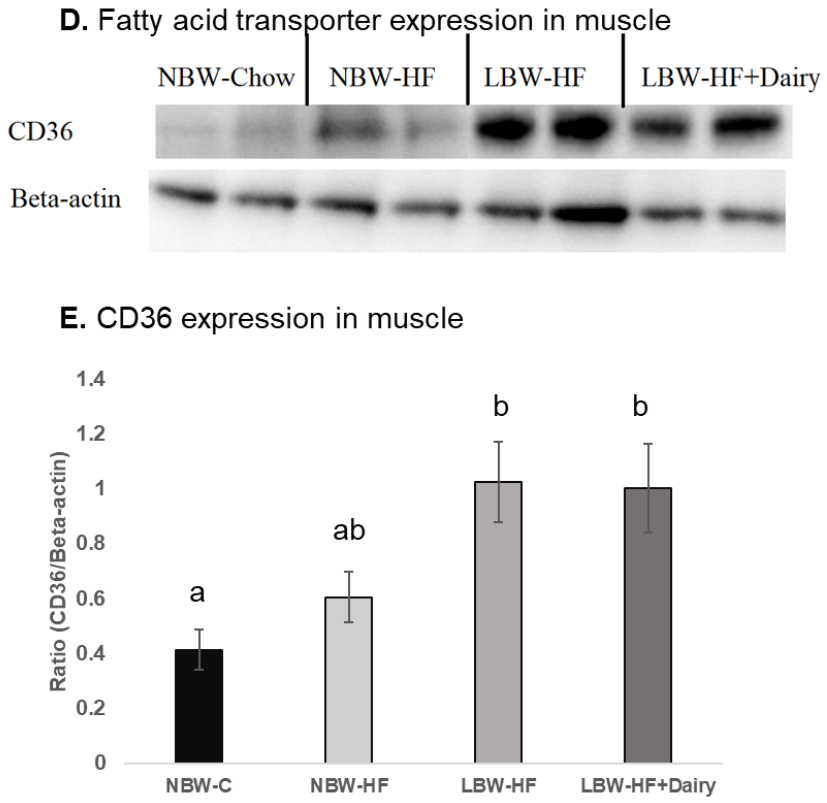


Figure 3-3. Expressions of CD36 and FABP2 relative to beta-actin in mucosal scrapings (**A, B, C**) and/or biceps femoris (**D, E**) in NBW and LBW swine fed experimental diets at 12-weeks of age. CD36: cluster of differentiation 36; NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

3.3.7 Carcass traits and meat quality

High fat feeding did not significantly change carcass traits or meat quality in NBW and LBW swine compared with Chow-fed NBW swine (**Table 3-6**). Feeding dairy products significantly decreased the subcutaneous fat depth, which further increased Canadian Lean Yield % in LBW swine fed a HF diet ($p<0.05$, **Table 3-6**). No significant difference was observed in other indices of pork quality such as the subjective pork marbling score.

Table 3-6. Carcass traits of NBW and LBW swine fed experimental diets at 12-week of age.

Carcass characteristics	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-value*
Hot carcass weight, kg	37.08 ± 1.66^{ab}	40.77 ± 1.28^{a}	36.26 ± 2.04^{ab}	33.44 ± 0.88^{b}	0.061
Subcutaneous fat depth, cm	1.21 ± 0.14^{ab}	1.45 ± 0.09^{a}	1.40 ± 0.14^{a}	0.90 ± 0.04^{b}	0.023
Loin muscle depth, cm	3.76 ± 0.38	4.62 ± 0.34	4.56 ± 0.25	4.62 ± 0.19	0.193
Canadian lean yield, %	62.51 ± 0.58^{ab}	61.69 ± 0.34^{a}	62.00 ± 0.72^{a}	64.79 ± 0.34^{b}	0.008
Intramuscular fat, %	1.59 ± 0.10	2.40 ± 0.28	2.37 ± 0.26	2.51 ± 0.33	0.121
Subjective pork marbling score	1.40 ± 0.24	2.17 ± 0.4	2.00 ± 0.27	1.80 ± 0.37	0.280

Values are shown as means ±SE. Canadian lean yield (%) = 68.1863-(0.7833 × fat depth) + (0.0689 × muscle depth)+(0.0080 × fat depth^2)-(0.0002 × muscle depth^2)+(0.0006 × fat depth × muscle depth) (Pomar and Marcoux, 2003). NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

3.4 Discussion

In the present study, we utilized a LBW swine model of IR to investigate the molecular transport pathway on intestinal and muscle lipid metabolism. The increased intestinal lipid absorption and muscle steatosis in LBW swine are likely explained by the upregulation of CD36. We also assessed the impact of dairy intake on metabolic consequences and confirmed that LBW piglets consuming 3 servings/day of full-fat dairy products (compared to HF-diet alone) exhibited

improved insulin sensitivity, enhanced incorporation of dietary long-chain polyunsaturated fatty acids into pork, as well as increased predicted lean yield of the carcass.

3.4.1 Early insulin resistance induced by HF diet can be ameliorated by dairy intake.

Low birth weight infants have been shown to have an increased risk of developing MetS later in life (Ferrie et al., 2006; de Mendonça et al., 2020). Animal studies also suggest that LBW rodents or piglets are more susceptible to hypertension, increased pro-inflammatory cytokines, and/or IR during adulthood (Barnett et al., 2017; Fontaine et al., 2019). Our study is consistent with these findings further highlighting that LBW piglets can be susceptible to develop fasting hyperglycemia and hyperinsulinemia (early IR) when consuming a HF diet. Additionally, our findings also support potential inverse association between dairy intake and IR in LBW swine. Ruminant-derived dairy products contain a diverse fatty acid profile, which mainly consists of short-chain (C4:0) and medium-chain fatty acids (C6:0 to C12:0), myristic acid (C14:0), palmitic acid (C16:0), monounsaturated fatty acids (MUFAs) (i.e. oleic acid, C18:1n9), polyunsaturated fatty acids (PUFAs) and unique odd-chain fatty acids (i.e. C15:0 and C17:0) (Abdullah et al., 2015). Butyric acid (C4:0) and myristic acid (C14:0) have been shown to improve insulin sensitivity in mice (Amine et al., 2021; Gao et al., 2009; Takato et al., 2017). Odd-chain fatty acids such as pentadecanoic acid (C15:0) and margaric acid (C17:0) have also been found to lower the risk of type II diabetes and total mortality in humans (Zhuang et al., 2019; Imamura et al., 2018). Furthermore, Vitamin K2 (which can be uniquely synthesized by bacteria from cheese production), may also ameliorate IR by potentially modulating pro-inflammatory cytokine levels (Struijk et al., 2013; Shea et al., 2008). The dietary intake of menaquinones (Vitamin K2) has also been inversely associated with the risk of type II diabetes (Beulens et al., 2010).

3.4.2 Incorporation of dietary-derived fatty acids in the muscle tissue (pork).

The high fat diet used in this study was found to cause muscle steatosis in LBW swine and is consistent with the previous studies using the same approach (Fontaine et al., 2019). To further identify fatty acids incorporation, we analyzed the fatty acids composition in both the plasma and muscle via gas chromatography. We know that increasing dietary fat intake can alter fatty acid composition in meat. We also highlight that despite fishmeal being used as a source of long-chain PUFA, it did not significantly elevate EPA, DPA or DHA level in muscle in either NBW or LBW swine fed a HF diet compared with NBW-Chow group. However, we did find that dairy products raised DPA and DHA in muscle of LBW swine, suggesting a preferential incorporation of long-chain fatty acids into the muscle. Of relevance to this, an earlier study has indicated that feeding dairy may potentially enhance long-chain omega-3 fatty acids incorporation in tissues, provided that alpha-linoleic acid (ALA, C18:3n3) supply from the diet is sufficient (Drouin et al., 2018). For instance, myristic acid (C14:0) has been found to activate delta-6 desaturase in cultured rat liver cells, and delta-6 desaturase is one of the rate-limiting enzymes involved in the synthesis of long-chain PUFA (Jan et al., 2004). Additionally, fatty acids shunted to mitochondrial beta-oxidation are based on the number of carbon and double bonds with the rate being reported as follows: short-chain fatty acids > medium-chain fatty acids > ALA (DeLany et al., 2000; Leyton et al., 1987). Despite ALA exhibiting a high affinity for the beta-oxidation, short- and medium- chain fatty acids have even higher priority for the oxidation pathway and can spare ALA from being oxidized. It is also thought the spared ALA can be re-directed towards the conversion pathway of long-chain PUFA (Drouin et al., 2018). Consequently, we speculate here that long-chain omega 3 fatty acid synthesis pathway could be indirectly influenced by the addition of dairy products.

3.4.3 Fatty acid transporter candidates

We hypothesized that preferential increased lipid deposition in muscle tissue as well as the corresponding elevated intestinal lipid absorption in LBW swine could be due to the upregulation of fatty acid transporters or relevant proteins such as: CD36 (expressed in skeletal muscle and small intestine, regulating the uptake of long-chain fatty acids); FATP4 (expressed on the brush border membrane of the small intestine to transport fatty acids); FABP2 (expressed in the epithelial cells of the mucosal layer in small intestine, involved in fatty acid uptake); or VAMP7 (involved in the PCTV trafficking together with CD36 in the small intestine) (Lewis et al., 2015; Glatz & Luiken. 2017; Siddiqi et al., 2010; Said, 2018). We found a significant elevation of both intestinal and muscle CD36 (but not FABP2) in LBW swine under a HF diet. Intestinal CD36 expression at mRNA level was not significantly different between NBW-HF and LBW-HF as described in a previous study using the same approach (Fontaine et al., 2019). However, CD36 protein levels were significantly different between these two groups, suggesting a potential post-transcriptional modification. Studies in mice indicate that the phenotype of the FABP2 knockout displayed similar fecal lipid content compared with wild-type when fed a HF diet, implying that FABP2 may not be essential for fatty acid uptake in the small intestine (Lagakos et al., 2011; Gajda et al., 2013; Lackey et al., 2020). In contrast, CD36 knockout mice have impaired fat absorption and decreased chylomicron production in the proximal segment of small intestine with no significant compensatory pathways found, suggesting a predominant role in intestinal lipid absorption (Nassir et al., 2007). CD36 has also been shown to be the fundamental membrane protein involved in the long-chain fatty acid uptake into skeletal myocytes, which results from the activation of peroxisome proliferator-activated receptors (PPARs) induced by specific fatty acids (Glatz & Luiken. 2017). Overexpression of muscle-specific PPARα in a transgenic mouse model led to a five-fold increase in CD36 gene expression

(Finick et al., 2005). CD36 is transferred from endosomes to sarcolemma to facilitate fatty acid uptake in muscle cells, induced by muscle contraction or insulin (Luiken et al., 2002; Luiken et al., 2003). Hyperglycemia may also contribute to muscle steatosis by increasing de novo lipogenesis activity in muscle, which further triggers abnormal CD36 translocation (Savage et al., 2007). Collectively, our data supports consistent findings of the important role of CD36 in intestinal and muscle lipid metabolism.

3.4.4 The impact of dairy products on carcass traits

We speculate that the preferential muscle lipid uptake in LBW-HF+Dairy swine may potentially be at the expense of decreasing fat content in adipose tissue (as we have seen a decreased subcutaneous fat thickness). This in turn may further indirectly elevate the Canadian Lean Yield %. Lean yield content is one of the factors determining pork carcass value in the Canadian grading system, which can be predicted via backfat and loin muscle depth measurements (Pomar and Marcoux, 2003). Low birth weight swine potentially exhibit severe growth retardation (Gondret et al., 2005; Rehfeldt and Kuhn, 2006; Yan et al., 2017). Interestingly, it was notable that dairy products in this study compensated the shortcoming in growth performance of LBW swine by increasing the predicted lean yield. Leucine has been found to enhance myogenesis in skeletal muscle in piglets (Wan et al., 2016). Dairy products are one of the good sources of essential amino acids and contained more leucine than traditional plant-based diet (soybean or wheat) (Gorissen et al., 2018). Increased leanness may improve the carcass grading index, as well as increasing the economic value (Pomar et al., 2009). This finding may suggest the importance of bioactive nutrients (such as those from the dairy) to improve the LBW carcass value in swine.

3.5 Conclusions

In summary, findings from this study provide evidence on the mechanistic pathway in intestinal and muscle lipid metabolism in an innovative LBW swine model. We have learned that increased intestinal lipid absorption and preferential muscle lipid deposition in LBW swine can be explained by the upregulation of CD36. We have also demonstrated that increasing dairy intake can enhance the incorporation of dietary long-chain PUFA into pork, as well as increasing the predicted lean yield of the carcass.

CHAPTER 4. Low birth weight swine did not exhibit major defects on growth performance, meat quality or carcass traits

This Chapter is an adaptation of a research paper that is in preparation and will be submitted to *Canadian Journal of Animal Science*.

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4.1 Background

Livestock animals that have large litter sizes can exhibit severe naturally occurring intrauterine-growth restriction (Wu et al., 2006). Impaired uterine capacity, nutrient supply and blood flow affect fetal growth, which leads to neonates born with low birth weight (LBW) (Chen et al., 2021). During the past several decades, the litter size of newborn piglets has been increasing owing to advanced breeding technologies and genetic selection, resulting in a common LBW trend (less than 1.0 kg at birth) (Wu et al., 2006). Birth weight has an underlying influence on nutrient absorption due to reduced gut capacity and impaired secretion of digestive enzymes, which can all affect growth performance (Wellington et al., 2021). Low birth weight is also associated with increased risk of neonatal morbidity and mortality (Chen et al., 2021). Studies have investigated the influence of birth weight on postnatal growth, carcass characteristics and meat quality in swine with mixed results. Piglets born with LBW have been shown to exhibit

reduced postnatal growth rate with lower average daily gain compared with normal birth weight (NBW) littermates (Gondret et al., 2005; Rehfeldt and Kuhn, 2006; Fontaine et al., 2019). Slower growth also affects the development of muscle fibers, as a lower total number yet increased cross-sectional area of fibers have been seen in LBW piglets (Gondret et al., 2005; Prado et al., 2013). Pork and carcass from LBW swine have been shown to have unfavorable consumer trails such as higher pH, drip loss, discoloration and/or decreased tenderness (Gondret et al., 2006; Matyba et al., 2021, Li et al., 2017[2]; Liu et al., 2014). Collagen is one of factors associated with meat tenderness, and LBW pigs can exhibit increased abundance, leading to increased meat toughness (Gondret et al., 2006). These observations suggested that LBW piglets may not be considered as full-value market hogs. However, there are also some studies that suggest that birth weight is not related to detrimental effects on meat quality (Alvarenga et al., 2014; Sundrum et al., 2021, Ceron et al., 2021, Beaulieu et al., 2010). To advance this field, the present study was conducted to further elucidate the impact of birth weight on growth performance, carcass traits and meat quality, as well as muscle fibers and collagen content in *longissimus* muscle. Additionally, average daily gain has been found to be linearly associated with increasing levels of lactose and/or whey protein concentrate in weaning piglets, often more so than simple production diets (Yun et al., 2005; Cromwell et al., 2008). Therefore, another objective of this study was to determine the impact of feeding increased dairy products on postnatal growth and overall meat quality.

In **Chapter 3**, we have discussed the impact of birth weight on metabolic changes in intestinal and muscle lipid metabolism. In this Chapter, we subsequently hypothesized that LBW swine might exhibit unfavorable changes on postnatal growth and overall meat quality compared to

NBW counterparts. We also proposed that consuming 3 servings/day of full-fat dairy products might potentially compensate for any growth defects observed in LBW swine.

4.2 Study designs and Methodology

4.2.1 Animal and housing:

Details for study design and experimental diets have been described in **Chapter 3**. Briefly, full-term newborn male piglets were weighed and selected based on NBW and LBW. At 5-weeks of age, NBW and LBW piglets were fed a 1-week transition diet, then were randomly assigned to three experimental diets: 1-chow diet, 2-HF diet or 3-HF diet supplemented with 3 servings per 2000kcal/day of full fat dairy products (HF+Dairy). The groups were as follows: NBW-Chow (n=5), NBW-HF (n=6), LBW-HF (n=8) and LBW-HF+Dairy (n=5). Chow and HF diet were provided ad libitum. Both HF and HF +Dairy diets were isocaloric, and piglets on HF+Dairy diet were pair-fed to piglets on HF diet to ensure similar energy intake. The nutritional composition of chow and HF diets have been previously described (Fontaine et al., 2019). In short, HF diet consisted of 46%, 33% and 21% of total energies from fat (mainly lard), carbohydrates (mainly fructose) and protein respectively. 1% w/w cholesterol was added. On the contrary, chow diet consisted of 14%, 69% and 17% of total energies from fat, carbohydrates and protein respectively. Dairy extract (high-lactose whey) was added to support the growth of piglets. The specific nutritional composition of dairy products has recently been described in She et al. (2022). Servings of dairy products included: whole milk powder (3.25% fat, 33g/serving; Bulk Barn, Canada), plain yogurt (10% fat, 175ml/serving; Liberté, Canada) and mozzarella cheese (28% fat, 50g/serving; No Name®, Canada). Food intake and body weight were measured on a daily and weekly basis respectively until 12-week of age.

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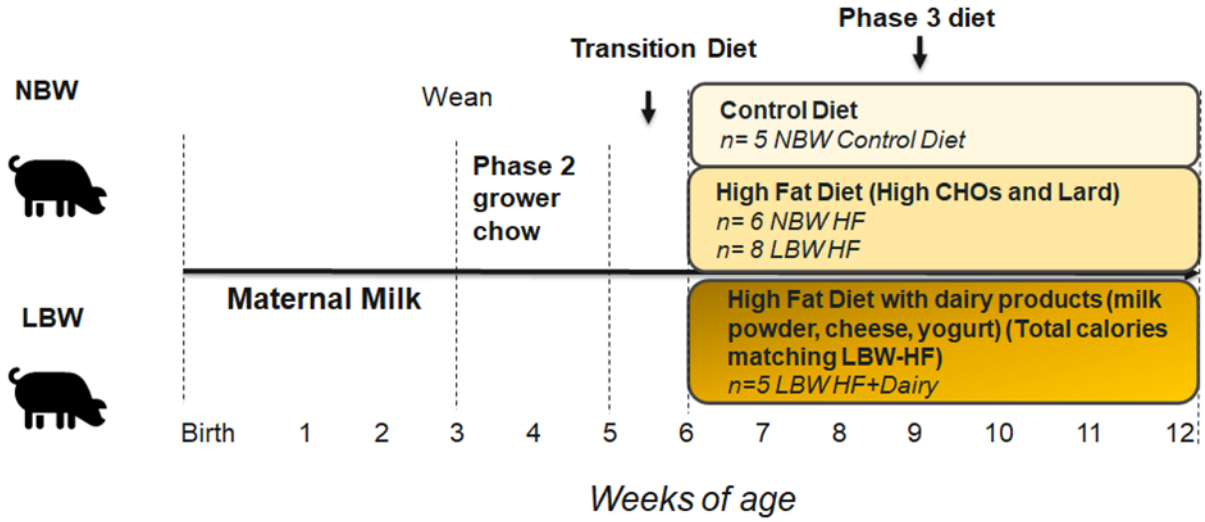

Figure 4-1. Study design to investigate growth, meat quality and carcass traits in LBW swine. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products

4.2.3 Slaughter and carcass traits

At 12-weeks of age, piglets were euthanized, bled and eviscerated by trained staff at SRTC. Carcasses were dehaired by flame and immediately transported to Agri-Food Discovery Place (University of Alberta, Edmonton, Canada). The hot carcass weight was recorded. The carcass then was stored in a 4°C cold room. After 24 hours, the cold carcass weight and carcass length (from the first cervical vertebrae bone atlas to base of the tail) were recorded. Each carcass was cut in half, and each side was used to dissect into primal cuts (pork shoulder, pork belly, pork loin and pork legs) according to Meat Cuts Manual, Canadian Food Inspection Agency. The weight was recorded as a percentage of hot carcass weight basis. **Figure 4-2.** shows the breakdown in loin eye muscle from the right side of the carcass for meat quality measurements. Subcutaneous fat depth and loin muscle depth were determined by using one chop from steak A

(**Figure 4-2**), which then were used to calculate Canadian Lean Yield % according to (Pomar and Marcoux, 2003).

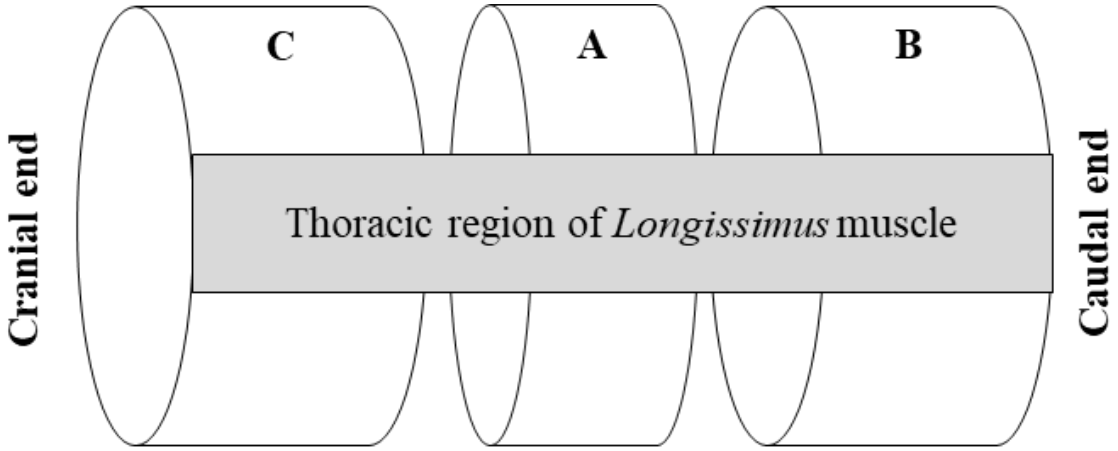

Figure 4-2. The breakdown of loin eye muscle from right side of the carcass (24 hr postmortem). Thoracic region A was used to determine meat color, subcutaneous fat depth and muscle depth. Region B was used for cooking, determining cooking loss and Warner-Bratzler shear force after cooking. Region C was used to determine muscle fiber typing. After samples were taken for muscle fiber measurement, epimysium was trimmed off from this chop. It was further cut into small cubes, frozen, lyophilized, grounded and used for proximate analysis and collagen measurement.

4.2.4 Muscle fibers

Muscle fiber type and histological and immune-histological characteristics of *longissimus* muscle were determined by using 1cm^3 muscle cube at 24-hr postmortem after immersing in dry-ice cooled acetone as described in Roy et al. (2018). Cubes were sectioned transversely (10 µm thick) with muscle fiber direction in a cryostat (Leica CM1850 cryostat, Leica Biosystems Nussloch, Germany) at -25 ℃ and serial sections were mounted on dry slide glass and store at -80 ℃ until staining. Staining was performed by removing mounted muscle sections from storage

and air drying them at room temperature for 30 mins. Muscle fiber type was analyzed by using the myofibrillar adenosine triphosphatase (mATPase) staining method (Brooke and Kaiser, 1970) and the nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase (NADH-TR) staining method (Roy et al., 2018). Type I muscle fibers were detected by immuno-fluorescence histochemistry using monoclonal antibodies (S58, skeletal muscle myosin antibody from Santa Cruz Biotechnology, Inc.) specific to the type I myosin isoform (Roy et al., 2018). Muscle sections were also pre-incubated in acid (pH 4.3) and alkali (pH 10.5) before staining for mATPase. Image J was used to measure cross-sectional areas of muscle fibers.

4.2.5 Meat quality

Loin eye muscle from the right side of the carcass was used for the following meat quality measurements.

1. Meat color

A freshly cut cross-sectional surface of chops from *longissimus* muscle (avoiding intramuscular fat or connective tissue) was used to determine meet color after being bloomed for 20 mins at room temperature. CR-400 chroma meter (Konica Minolta Sensing, Inc., Japan) in CIE standard color system was used to measure lightness (L*; ranging from 0 for black to 100 for ideal white), redness (a*; color coordinate where positive indicates redness and negative indicates greenness), yellowness (b*; color coordinates where positive indicates yellowness and negative indicates blueness) and color intensity or saturation (C*; chroma and hue) (Billmeyer, 1988). The diameter of the aperture area was 8 mm with a 2° standard observer angle, and the D65 illuminant setting was used. A white calibration ceramic tile provided by the manufacturer was used to calibrate the chroma meter before measurements and the readings of the standard white tile were L* = 93.21,

a* = 0.3126, b* = 3192. Each chop was measured in triplicate with mean value used for statistical analysis.

2. Ultimate pH (24hrs)

Ultimate pH at 24 hrs was measured by an Accumet AP71 pH meter (Fisher Scientific, Mississauga, Ontario). The pH electrode was temperature compensated (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON) and was calibrated with standard pH 4.0 and 7.0 buffers. A knife was used to open a small cut in muscle, followed by inserting the electrode. pH was measured in triplicate with mean value used for statistical analysis.

3. Drip loss

Drip loss was measured using the standard bag method (Honikel and Hamm, 1994). 50~60g *longissimus* muscle after epimysium being trimmed off was suspended in an inflated plastic bag with a metal hook for 24 hours at 4℃. Drip loss was calculated as weight loss on a percentage of initial weight of the muscle.

4. Cooking loss

A thermocouple (Tiny-Tag View 2S, Gemini Data Loggers, Chichester, West Sussex, UK) was used to penetrate the steak at the geometrical centre to monitor the temperature change. The steak was cooked on an electric clam-shell style grill (General Electric 4 in 1 Grill/Griddle, China) at 165 ℃ (325 °F) until the inner temperature reached 71℃. After reaching designated temperature, cooking was stopped and the steak was removed from the grill, wrapped in a plastic bag and immersed in ice-cold water. Excessive water from evaporation or condensation was poured out and the steak was stored overnight at 4℃. On the second day, the steak was patted dry using paper towels and weighed. The equation to determined cooking loss is as follows:

$$Cooking\ loss\ (\%) = \frac{(original\ weight\ before\ cooking - weight\ after\ cooking\ and\ pat\ drying)}{original\ weight\ before\ cooking} * 100\%$$

5. Warner-Bratzler shear force

Measurements for Warner-Bratzler shear force have been described previously in Roy et al. (2018) previously. Briefly, six cores (1.27cm diameter) parallel to the direction of muscle fibers were prepared from each cooked steak using a metal cork drill. Visible fat and connective tissue were not selected. Mean value from six recorded peak force was calculated for each steak and was used for statistical analysis.

4.2.6 Proximate analysis

1. Moisture

The method for moisture measurements was adapted from Association of Official Agricultural Chemists (AOAC) (1990). Around 100 g of raw steak was taken and recorded with epimysium trimmed off. It was cut into small cubes and evenly distributed on an aluminum tray, covered with aluminum foil and frozen at -20℃ until lyophilized for 5-7days. The weight loss on a percentage of total raw meat weight was recorded as moisture content.

2. Crude fat

The method for crude fat (intramuscular fat) measurements has been previously described in Roy et al. (2018). Briefly, around 2 g of lyophilized steak was selected and weighed with epimysium and extra fat being trimmed off. After fat extraction, crude fat was calculated on a percentage of raw meat basis.

3. Crude protein

The method for crude protein measurements was adapted from AOAC (1990). Around 0.1g of lyophilized steak was selected and weighed. After hydrolyzation, neutralization and titration, crude protein content was calculated using a nitrogen-to-protein conversion factor of 6.25.

4. Ash

The method for ash measurements was adapted from AOAC (1990). Around 2g of lyophilized steak was selected, weighed and pre-dried. The steak was put in a glass bottle, heated at 110℃ for 24 hrs in an oven and incinerated at 490℃ for 24 hrs in a furnace. The remaining content after incineration was ash and calculated on a percentage of raw meat basis.

4.2.7 Collagen content

Details to measure total, soluble and insoluble collagen contents in meat have been previously described in Bruce et al. (2022).

1. Separation of soluble and insoluble collagen

The method to separate soluble and insoluble collagen was adapted from Hill (1966). Briefly, around 2 g of lyophilized meat was selected, weighed and heated. After cooling to room temperature, the supernatant (soluble collagen) and residue (insoluble collagen) were separated by centrifugation (3500g, 10mins). The quantification for each collagen will be described in the following section. Each extraction was performed in duplicate.

2. Acid hydrolysis

Aliquots of supernatant and residue were hydrolyzed in concentrated hydrochloric acid, followed by filtration, drying, reconstitution (in double distilled water) and neutralization (with sodium hydroxide). The neutralized samples were dried again and reconstituted in water, stored at -20℃ for the hydroxyproline assay.

3. Hydroxyproline assay

The hydroxyproline assay was used to determine collagen content, which was described by Bergman and Loxley (1963). Briefly, around 1ml of hydrolyte (soluble or insoluble) was selected, and a standard curve to measure hydroxyproline concentrations was established. The hydroxyproline concentration for each sample was determined by the standard curve, which further was converted to collagen concentration by using the conversion factor of 7.14 (Stanton and Light, 1987). Total collagen was calculated by the addition of both soluble and insoluble collagen. Collagen solubility was calculated by dividing the soluble collagen by total collagen and multiplying the result by 100%. Each sample was measured in duplication, and the mean value was used for statistical analysis.

4.2.8 Statistical analysis

Data are reported as mean±SEM. IBM SPSS (version 26.0) was used for statistical analysis. Data were analyzed by one-way ANOVA with diet as the main effect and in cases where a p-model was significant, post-hoc comparison tests was performed with the Tukey's HSD adjustment for multiple comparisons among groups. All tests with p-value <0.05 were considered statistically significant.

4.3 Results

4.3.1 Growth performance and food intake

As expected, all LBW piglets had significantly lower birth weight than NBW piglets ($p<0.05$, **Table 4-1**). Chow-fed NBW piglets had significantly lower average daily energy intake and energy to gain ratio, compared with other HF-fed groups ($p<0.05$, **Table 4-1)**. LBW swine had a body weight significantly lower than NBW swine from birth to 5-weeks of age (before the start of the dietary intervention) (**Table 4-2**). However, from 6- to 12-weeks of age (during dietary

intervention), body weight differences were not statistically significant (**Table 4-2**). No other significant differences were found in terms of average daily gain and final body weight among all groups.

Table 4-1. Growth performance and feed consumption of NBW and LBW swine fed experimental diets.

	NBW-Chow	**NBW-HF**	**LBW-HF**	**LBW-HF+Dairy**	***P-value***
Birth weight, kg	1.620±0.066[a]	1.733±0.095[a]	1.228±0.081[b]	1.225±0.065[b]	<0.001
Final weight, kg	48.44±3.164	53.62±1.970	48.10±2.771	45.64 ± 1.22	0.10
Average daily gain, g/d	779.2±47.34	854.0±15.80	794.7±41.00	753.2±49.4	0.30
Average daily energy intake, kcal/d	4076.9±290[a]	5716.7±340[b]	5313.5±238[b]	5239.9±52.3[b]	<0.01
Energy to gain ratio	5.23±0.14[a]	6.67±0.29[b]	6.72±0.15[b]	6.98±0.22[b]	<0.01

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

Table 4-2. Weekly body weight of NBW and LBW swine.

Age (weeks)	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-value*
0 (at birth)	1.62 ± 0.07a	1.73 ± 0.09a	1.29 ± 0.08b	1.18 ± 0.06b	<0.01
1	2.94 ± 0.30 ab	3.57 ± 0.39a	2.50± 0.33ab	2.10 ± 0.16b	<0.05
2	4.5 ± 0.34ab	5.52 ± 0.59a	3.75 ± 0.49b	3.74 ± 0.21ab	<0.05
3	6.58 ± 0.29ab	7.52 ± 0.43a	5.65 ± 0.57b	5.36 ± 0.40b	<0.05
4	9.08 ± 0.74ab	9.95 ± 0.42a	7.31 ± 0.81b	6.24 ± 0.13b	<0.01
5	11.22 ± 1.23	13.05 ± 1.17	9.51 ± 1.03	8.90 ± 0.32	0.0502
6	14.44 ± 1.57	15.33 ± 1.70	12.51 ± 1.46	12.40 ± 0.52	0.4237
7	19.60 ± 1.64	21.52 ± 1.87	16.91 ± 1.90	15.70 ± 0.70	0.1252
8	25.92 ± 2.03	28.87 ± 2.10	23.95 ± 2.02	21.12 ± 1.23	0.0884
9	32.36 ± 2.08	35.38 ± 2.21	29.57 ± 2.40	26.82 ± 1.10	0.0788
10	37.66 ± 2.27	43.48 ± 2.54	37.08 ± 2.73	32.54 ± 1.21	0.0537
11	42.78 ± 2.93	46.85 ± 2.29	41.59 ± 2.71	37.34 ± 1.25	0.1223
12	48.44±3.16	53.62±1.97	48.10±2.77	45.64 ± 1.22	0.0960

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products. Week 1- week 4 (light shaded): before the dietary intervention. Week 5 (blue shaded): transition period. Week 6 – week 12 (dark shaded): during the intervention (experimental diets were provided).

4.3.2 Muscle fibers from longissimus thoracis muscle at 24 hr postmortem

Histochemistry and immunochemistry of muscle fiber typing, and frequency distribution for Type I, Type IIA and Type IIB and mean muscle fibers have been described in **Appendix C**. Birth weight and HF diet did not significantly change the diameter of Type I, Type IIA and Type IIB muscle fibers (**Table 4-3**). However, LBW-HF swine tended to have increased diameter of

Type I muscle fiber and mean diameter of muscle fibers compared with NBW swine fed either Chow diet or HF diet. Feeding dairy to LBW piglets increased this trend and was statistically significant at $p<0.05$ compared to NBW piglets (**Table 4-3**).

Table 4-3. Muscle fibers from longissimus thoracis muscle at 24 hr postmortem of NBW and LBW swine fed experimental diets at 12-week of age

	NBW-Chow	**NBW-HF**	**LBW-HF**	**LBW-HF+Dairy**	***P-value***
Type I diameter (µm)	38.86±0.88ab	37.36±1.58b	43.21±1.95ab	44.86±2.70a	0.040
Type IIA diameter (µm)	38.91±3.64	36.30±1.24	39.38±1.88	40.85±1.70	0.538
Type IIB diameter (µm)	49.94±2.80	45.85±1.29	51.08±2.63	53.25±1.82	0.172
Mean diameter (µm)	42.57±2.28a	39.83±1.23a	46.76±2.24ab	51.03±1.93b	0.006
Type I (%)	10.32±2.10	9.27±2.00	9.66±1.36	6.05±2.10	0.264
Type IIA (%)	16.40 ± 3.10	19.04 ± 2.95	17.00 ± 2.00	18.03 ± 3.10	0.844
Type IIB (%)	73.28 ± 3.41	71.69 ± 3.24	73.34 ± 2.20	75.92 ± 3.41	0.697
Total muscle fibers in loin muscle area	79152 ± 20129	128875 ± 19125	87888 ±12993	95396 ±20129	0.1073

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

4.3.3 Carcass traits

Birth weight and HF feeding did not significantly change any carcass traits in NBW and LBW swine (**Table 4-4**). Feeding dairy products as a part of HF diet tended to lower the hot carcass weight in LBW swine compared with HF-fed NBW swine ($p=0.061$, **Table 4-4**). Dairy products also significantly decreased the subcutaneous fat depth, which further increased Canadian Lean Yield % compared with LBW swine fed a HF diet ($p<0.05$, **Table 4-4**). LBW swine fed a HF diet tended to have increased pork loin weight %, rib end % and rear tender end % (on a hot carcass weight basis) compared with NBW swine fed either a Chow or HF diet, whereas feeding dairy products led to a significant increase compared to NBW piglets ($p<0.05$, **Table 4-4**). No other significant differences were found in terms of carcass traits such as cold carcass weight, carcass length, muscle depth, etc. among all groups.

Table 4-4. Carcass traits at 24 hr postmortem of NBW and LBW swine fed experimental diets at 12-week of age

	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-value*
Hot carcass weight (kg)	37.08±1.66ab	40.77±1.28a	36.26±2.04ab	33.44±0.88b	0.061
Cold carcass weight (kg)	34.84±2.29	39.07±1.69	35.23±2.00	33.26±1.00	0.225
Carcass length (cm)	77.5±2.81	79.68±1.10	75.78±1.17	76.00±1.00	0.275
Subcutaneous fat depth (cm)	1.21 ± 0.17ab	1.45 ± 0.16a	1.40 ± 0.10a	0.90 ± 0.17b	0.023
Longissimus muscle depth (cm)	3.76 ± 0.42	4.62 ± 0.39	4.56 ± 0.26	4.56 ± 0.42	0.193
Canadian Lean Yield (%)	62.51±0.58ab	61.69±0.34a	62.00±0.72a	64.79±0.34b	0.008
Pork shoulder weight (%) HCW basis	29.99±1.14	30.11±0.75	26.88±1.68	27.50±1.93	0.308
Pork loin weight (%) HCW basis	21.70±0.62a	22.99±0.80a	29.22±2.92ab	31.97±2.08b	0.015
Pork leg weight (%) HCW basis	25.33±0.91	25.07±0.91	22.27±0.95	22.79±1.92	0.177
Pork belly weight (%) HCW basis	16.77±0.55	17.52±0.63	18.01±0.47	17.20±0.72	0.482
Rib end (%) HCW basis	1.81±0.07a	1.98±0.22a	3.09±0.65ab	3.91±0.26b	0.024
Rear tender end (%) HCW basis	2.19±0.20a	2.23±0.14a	3.51±0.47ab	4.21±0.23b	0.002
Center cut (%) HCW basis	7.15±0.64	7.42±0.29	8.38±0.75	9.83±1.20	0.129
Loin eye (%) HCW basis	0.58±0.05	0.61±0.05	0.62±0.04	0.65±0.05	0.746

Remainder (%) HCW basis	0.52±0.07	0.63±0.10	0.66±0.04	0.76±0.09	0.215

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. HCW: hot carcass weight; NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.
Canadian lean yield (%) = 68.1863 - (0.7833 × fat depth) + (0.0689 × muscle depth) + (0.0080 × fat depth^2) - (0.0002 × muscle depth^2) + (0.0006 × fat depth × muscle depth) (Pomar & Marcoux, 2003).

4.3.4 Meat quality from longissimus thoracis muscle at 24 hr postmortem

High fat feeding significantly lowered moisture content in NBW and LBW swine compared with NBW swine fed a Chow diet ($p<0.05$, **Table 4-5**). Feeding dairy products as a part of a HF diet in LBW swine increased moisture content to values no longer significantly different from NBW-Chow (**Table 4-5**). Dairy products tended to increase the yellowness in the pork from LBW swine compared with NBW swine fed a Chow diet ($p=0.059$, **Table 4-5**). Birth weight did not significantly change any meat quality traits between NBW and LBW swine.

Table 4-5. Meat quality from longissimus thoracis muscle at 24 hr postmortem in NBW and LBW swine fed experimental diets at 12-week of age

	NBW-Chow	NBW-HF	LBW-HF	LBW-HF+Dairy	*P-value*
Warner-Bratzler shear force (N)	31.65±3.75	32.54±3.13	29.84±2.33	26.55±1.45	0.259
Ultimate pH at 24hr	5.53±0.02	5.52±0.04	5.47±0.05	5.52±0.04	0.678
Lightness (L*)	50.17±0.61	53.41±1.41	52.12±0.70	52.83±0.50	0.131
Redness (a*)	8.14±0.32	8.32±0.27	8.25±0.38	8.08±0.32	0.964
Yellowness (b*)	0.94±0.14a	1.54±0.49ab	1.53±0.27ab	2.52±0.38b	0.059
Chroma	8.21±0.31	8.56±0.31	8.44±0.42	8.42±0.29	0.933
Hue	6.59±1.04	10.34±3.22	9.98±1.58	15.03±2.35	0.136
Crude protein (%)	20.97±0.29	21.11±0.08	21.08±0.19	20.86±0.13	0.795
Intramuscular fat (%)	1.59±0.10	2.40±0.28	2.37±0.26	2.51±0.33	0.121
Ash (%)	1.17±0.01	1.12±0.03	1.16±0.02	1.17±0.02	0.237
Moisture (%)	75.82±0.11a	74.67±0.26b	74.90±0.25b	75.11±0.24ab	0.023
Drip loss (%)	1.87±0.45	4.39±1.10	3.43±0.52	2.53±0.16	0.098
Cooking loss (%)	19.91±2.26	18.02±1.51	18.60±0.78	16.82±0.61	0.514

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

4.3.5 Collagen content from longissimus thoracis muscle at 24 hr postmortem

Birth weight and HF diet did not significantly change total collagen, soluble collagen, insoluble collagen or collagen solubility in muscle in NBW and LBW swine (**Table 4-6**). HF feeding trended to lower soluble collagen in muscle in either NBW or LBW swine compared to Chow-fed NBW swine, whereas dairy intake led to a significant decrease in LBW swine compared to NBW-Chow group (**Table 4-6**).

Table 4-6. Collagen content from longissimus thoracis muscle at 24 hr postmortem of NBW and LBW swine fed experimental diets at 12-week of age

	NBW-Chow	**NBW-HF**	**LBW-HF**	**LBW-HF+Dairy**	***P-value***
Total collagen (mg/ g raw meat)	3.57±0.17	3.20±0.66	2.72±0.14	2.40±0.17	0.176
Insoluble collagen (mg / g raw meat)	2.36±0.24	2.27±0.52	1.86±0.09	1.71±0.14	0.387
Soluble collagen (mg / g raw meat)	1.21±0.09a	0.93±0.20ab	0.86±0.06ab	0.69±0.06b	0.067
Collagen solubility (%)	35.24±3.65	28.87±5.01	31.6±1.52	28.63±1.88	0.504

Values are shown as means ±SEM. Groups that do not share similar letters are statistically significant according to the Tukey's HSD test for multiple comparisons. NBW: normal birth weight; LBW: low birth weight; HF: high fat diet; HF+Dairy: HF diet supplemented with dairy products.

4.4 Discussion

In the present study, we investigated the growth performance, meat quality and carcass traits in LBW swine. LBW swine fed a HF diet did not exhibit significant impairments in growth, pork pH, tenderness, color and carcass yield compared with NBW littermates. We also assessed the impact of feeding increased dairy on postnatal growth and overall meat quality and found that

consuming 3 servings/day of full-fat dairy products can potentially enhance Type I muscle fiber hypertrophy and increase the Canadian Lean Yield %.

4.4.1 Birth weight had limited effect on growth performance in the context of a high fat diet

The impact of LBW on postnatal growth performance in piglets has remained a critical issue in swine production industry (Gondret et al., 2005; Adjerid et al., 2021; Fontaine et al., 2019). It is commonly recognized that lower birth weight in piglets can be associated with smaller size at slaughter age, reducing economic return when compared to normal weight littermates. Some studies have reported that LBW piglets showed reduced postnatal growth rate when fed either a chow or HF diet within the first few months after birth (Gondret et al., 2005; Rehfeldt and Kuhn, 2006; Fontaine et al., 2019). Interestingly, results from the current study are not entirely consistent with these findings. Our data shows that LBW piglets were able to catch up to their NBW counterparts (fed an energy-dense diet) since growth performance parameters (final weight, average daily gain, average daily energy intake and weekly body weight) were all non-significant during the intervention. Prenatal undernutrition has been associated with LBW infants, and rapid change postnatally to sufficient or overnutrition has been proposed as one mechanism to explain the increased risk of developing metabolic syndrome (Li et al., 2017a). For instance, a rise in growth-related hormone levels such as insulin and insulin-like growth factor (IGF) have been observed in LBW infants, which could potentially explain the rapid growth in these infants catching up to NBW infants (Li et al., 2017a). Altogether, our findings suggest that in the context of overnutrition, LBW piglets were able to catch up to the NBW piglets.

4.4.2 Dairy products potentially enhanced the synthesis of muscle fibers

Muscle fiber (total number and cross-sectional area) is an important factor associated with swine postnatal growth performance. Pigs that grow faster usually have a greater numbers of muscle fibers than slow-growing littermates (Dwyer et al., 1993). Muscle fibers are formed during the prenatal development (myogenesis), which is very sensitive to maternal nutrition status (Tristan et al., 2009). Muscle fibers are further differentiated into slow-twitch (Type I) or fast-twitch (Type IIA, IIX & IIB) based on different ATPase activities (Listrat et al., 2016). The growth of skeletal muscle is severely impaired by intrauterine growth restriction in neonates, and LBW piglets exhibit less total number of muscle fibers or delayed muscle maturity at birth (Gondret et al., 2005; Prado et al., 2013). However, excessive postnatal nutrition will accelerate the muscle fiber growth by stimulating protein-synthesis gene expressions in LBW pigs (Liang et al.,2018). This has been observed in our data for which LBW swine fed a HF diet with dairy products showed increased diameter of slow-twitch Type I muscle fiber (hypertrophy) and greater mean diameter of muscle fibers. This may be partially explained by an improvement in mammalian target of rapamycin (mTOR) protein synthesis pathway (Liang et al.,2018). Leucine and its metabolite β-hydroxy-β-methylbutyrate have been found to play a critical role on stimulating skeletal muscle protein synthesis in piglets (Boutry et al., 2013; Wan et al., 2016). Feeding β-hydroxy-β-methylbutyrate in piglets elevated mRNA expressions of mTOR and IGF-1, which increased total amount of muscle tissue (Wan et al., 2016). Dairy products are excellent sources of essential amino acids and contains more leucine than traditional plant-based (soybean or wheat) diets (Rondanelli et al., 2020; Gorissen et al., 2018). Collectively, these findings suggest that adding dairy products to the feed, by providing more essential nutrients needed for myogenesis, may enhance the growth potential of LBW swine.

4.4.3 Birth weight had limited effect on carcass traits and meat quality in the context of a high fat diet

The impact of LBW to pork quality and carcass characteristics has also continued to be controversial. Some studies have reported that the pork or carcass yielded from LBW pigs exhibited unfavorable changes to pH, drip loss and tenderness, or showed reduced hot carcass weight, increased backfat thickness or decreased lean meat content (Gondret et al., 2006; Matyba et al., 2021, Li et al., 2017b; Liu et al., 2014). Other studies have suggested that birth weight had limited impact on pork quality or carcass weight (Alvarenga et al., 2014; Sundrum et al., 2021, Ceron et al., 2021, Beaulieu et al., 2010). Results from the current study are in agreement with Beulieu et al. (2010) and Alvarenga et al. (2014) that the birth weight does not significantly affect color (L*, a*, b*, chroma and Hue), ultimate pH, water-holding capacity (cooking loss and drip loss) or meat tenderness (Warner-Bratzler shear force) at 24-hr postmortem. Notably, LBW and/or HF-feeding may potentially cause an increase in lightness (L*) and Warner-Bratzler shear force (tenderness) or a decrease in pH at 45min postmortem (Liu et al., 2014; Alvarenga et al., 2014). Liu et al. (2014) suggested that LBW piglets had more L-lactase dehydrogenase than NBW piglets, which could increase the muscle glycolysis and contribute to a lower pH within a short time after being slaughtered, which may increase the risk of forming a pale, soft and exudative (PSE) meat. However, this phenomenon was not observed in LBW swine fed a HF diet in this study. It has been suggested that moisture content may decrease along with the decrease in birth weight or the increase in energy intake (Beulieu et al., 2010; Kang et al., 2020), which was found in this study. Interestingly, when dairy products were introduced, moisture content was increased in LBW swine. Barbut (2010) suggested that the addition of dairy proteins (i.e. milk powders, caseinate or whey proteins) may increase the moisture binding ability in

meat. Additionally, we further identified that birth weight did not have significant impact on carcass weight, lean yield or the yield from primal cuts (on a hot carcass weight basis), which disagrees with Gondret et al. (2006) but is congruent with Beulieu et al. (2010). Interestingly, feeding increased dairy improved the predicted lean yield and proportions of primal cuts from pork loin, rib end and rear tender end in LBW piglets. Increased lean yield may improve the LBW carcass value in the carcass grading system, which could generate more economic revenue for the swine industry (Pomar et al., 2009). Hence, our data supports the notion that meat from LBW swine may not always result in unfavorable consumer indices.

4.4.4 The impact of dairy products on meat tenderness

Meat tenderness can be objectively measured by Warner-Bratzler shear force and is associated with connective tissue, intramuscular fat and aging (proteolysis). (Starkey et al., 2017). Collagen is the primary protein found in connective tissue (Starkey et al., 2017). Matyba et al. (2021) and Gondret et al. (2006) reported that LBW or intrauterine growth restricted pigs contained more amount of total collagen in *longissimus* muscle compared with NBW counterparts, which potentially increases meat toughness. In the present study, we did not expect to find a difference in collagen content in HF-fed LBW or NBW swine, yet dairy-feeding tended to reduce soluble collagen. However, the mechanism for how dairy products affect collagen synthesis, breakdown or the formation of soluble form in pigs remains unclear.

Some studies have suggested that muscle fiber is negatively associated with meat tenderness by increasing muscle fiber length and reducing drip loss (Gondret et al., 2006; Koomkrong et al., 2017). Despite exhibiting longer diameter of Type I muscle fiber, the LBW-HF+Dairy group did not show any differences in meat tenderness or drip loss compared to other groups.

In our study, we used a 95% confidence interval (CI) to define NBW and LBW phenotypes. Piglets with birth weight below 95% CI were categorized as LBW, ranging from 0.8 kg to 1.4 kg. By contrast, other studies that indicate LBW meat demerits had a stricter definition with birth weight ranging from 0.8 kg to 1.1 kg (Gondret et al., 2005; Gondret et al., 2006), 0.65±0.07 kg (Matyba et al., 2021) or 0.95±0.03 kg (Liu et al., 2014). These piglets potentially have more susceptibility to developing metabolic syndrome or exhibiting changes in postnatal growth and meat quality. Swine phenotype/genotype, muscle type, dietary quality, as well as the source of fat and feeding period may all lead to divergences in carcass traits, meat quality or chemical composition.

4.5 Conclusion

In summary, findings from the present study provide new evidence that LBW piglets can potentially catch up to NBW littermates in the context of a HF diet, which is partially due to the hypertrophy of Type I muscle fiber. These data support the notion that depending on the LBW phenotype and feeding practice, pork quality may not necessarily be impaired. We also illustrate that dairy products may play an important role in postnatal myogenesis, as well as increasing the carcass yield in LBW swine.

CHAPTER 5. General discussion

5.1 Summary of findings

In both studies (Chapters 3 and 4), we utilized a low birth weight (LBW) swine model of insulin resistance (IR) to investigate intestinal and muscle lipid metabolism, and the impact of birth weight on growth performance, meat quality and carcass traits. We also assessed the effect of full-fat dairy products (milk, cheese and yogurt) on metabolic disorders (i.e. IR and dyslipidemia), postnatal growth and carcass yield in LBW swine.

In Chapter 3, we hypothesized that feeding increased dairy (3 servings/day) would reduce IR in LBW swine. Our data showed that LBW swine fed a high fat (HF) diet can exhibit early signs of IR (elevated fasting glucose, fasting insulin and HOMA-IR) compared to NBW-Chow group. We confirmed that dairy products improved insulin sensitivity by lowering these levels to values no longer significantly different from NBW-Chow. We also hypothesized that LBW swine might exhibit elevated triglyceride levels in plasma and muscle. Protein expression of candidate transporters (CD36, FABP2, FATP4 and VAMP7) in enterocytes and/or myocytes in LBW swine could potentially explain the development of hypertriglyceridemia and ectopic lipotoxicity. We demonstrated that HF diet elevated muscle triglyceride (TG) level in LBW swine compared to NBW-Chow and confirmed that CD36 (but not FABP2) was elevated in both muscle and small intestine in LBW swine (regardless of the dairy intake). Additionally, we proposed that increasing dietary intake of dairy-derived fatty acids would result in higher proportion of these fatty acids being incorporated into the muscle, likely facilitated by candidate fatty acid transporters. We confirmed that increased dairy intake (as a part of HF diet) preferentially increased the incorporation of dairy fat (C14:0 & C17:0) and long-chain polyunsaturated fatty acids (PUFA) (DPA & DHA) in muscle in LBW swine.

In Chapter 4, we hypothesized that LBW swine would exhibit unfavorable changes in postnatal growth, pork quality or carcass characteristics compared to NBW counterparts. Interestingly, weekly body weight, average daily gain, average daily energy intake and final weight of LBW piglets were all non-significant compared with NBW littermates, nor did we find any differences in overall pork/carcass quality (i.e. tenderness, color, pH and carcass yield). We also proposed that increased dairy intake might compensate for growth impairment in LBW swine, possibly improving pork quality. Results revealed that dairy products enlarged the diameter of Type I muscle fiber and increased the carcass lean yield in LBW swine, which can potentially increase the carcass value.

5.2 General discussion and implications

5.2.1 The effect of dairy on preferentially increased incorporation of long chain PUFA in muscle

We primarily utilized fishmeal (120g/kg as part of the HF diet) as a source of long chain PUFA (and protein), however interestingly HF-fed swine (either NBW or LBW) did not contain more omega 3 fatty acids in muscle compared with Chow-fed NBW swine (**Chapter 3**). In the LBW-HF+Dairy group, around a quarter of HF meal was replaced by dairy, resulting in less fishmeal intake, yet LBW-HF+Dairy group contained more DPA and DHA than LBW-HF group. It has been suggested that increased dairy intake may potentially enhance the incorporation of long chain PUFA in tissues, on the premise that the diet provides sufficient ALA (C18:3n3) (Drouin et al., 2018). ALA and delta-6 desaturase are the main substrate/enzymes involved in the synthesis of long-chain PUFA (Jan et al., 2004; Drouin et al., 2018). Dairy fat such as myristic acid (C14:0) can activate delta-6 desaturase (Jan et al., 2004). ALA can exhibit a high affinity for the mitochondrial beta-oxidation, yet short/medium chain

fatty acids have even higher priority for the oxidation pathway due to lower number of carbon and less double bonds (priority for beta-oxidation is: short chain fatty acids > medium chain fatty acids > ALA) (DeLany et al., 2000; Leyton et al., 1987). It is thought that the ALA can be spared from the oxidation due to short/medium chain fatty acids, and the spared ALA can be re-directed towards the conversion pathway of long-chain PUFA (Drouin et al., 2018). As a result, dairy products could indirectly impact the long-chain omega 3 fatty acid synthesis pathway. It has also been suggested that fatty acid transporters such as CD36 can preferentially favor long-chain PUFA absorption in small intestine and deposition in muscle, which could partially explain the higher proportion of long-chain PUFA found in muscle (Dash et al., 2015).

We have shown in this study that not all saturated fatty acids have detrimental effects on health. Dairy fat, for instance, can not only improve IR but also potentially enhance PUFA synthesis. These data (along with others) support the inclusion of full fat dairy as part of a healthy diet. It is notable that the current version of Canada Food Guide does not necessarily support this recommendation.

5.2.2 Fatty acid transporters and their roles in intestinal and muscle lipid metabolism

CD36, FABP2 and FATP4 are present on the cell membrane of enterocytes and/or myocytes, facilitating lipid (mainly long-chain fatty acids) uptake into tissues (Dash et al., 2015; Glatz & Luiken. 2017; Said, 2018). VAMP7 and CD36 are involved in the formation of pre-chylomicron transport vesicle (PCTV) in the small intestine (Cifarelli and Abumrad, 2018; Siddiqi et al., 2010; Giammanco et al., 2015). A high fat, high fructose meal is believed to increase chylomicron synthesis, elevate postprandial plasma TG concentration and potentially cause ectopic lipotoxicity (i.e. muscle steatosis) (Giammanco et al., 2015; Desmarchelier et al., 2019). Due to challenges on availability of antibodies, several antibodies did not react with swine

samples, making them difficult to quantify (i.e. FATP4 and VAMP7). We found an upregulation of CD36 (but not FABP2) in small intestine and muscle in LBW swine compared with NBW swine. We also illustrated in **Chapter 3** that our findings support the hypothesis that CD36 plays a critical role in intestinal lipid absorption and lipid uptake (especially the preferential long-chain fatty acids uptake) in muscle. However, CD36 is not the only transporter involved in intestinal lipid absorption and/or muscle lipid deposition. FABP1 and FATP4 are highly expressed in small intestine, and FATP1 is highly expressed in skeletal muscle. It would be worth measuring protein expressions of these candidate transporters to see if they contribute to aberrant intestinal/muscle lipid metabolism.

5.2.3 The underlying application of dairy products in pork industry

The production of innovative omega-3 enriched pork has been intensively discussed in recent years. In this thesis, we have found that feeding increased dairy enhanced long-chain PUFA incorporation in *biceps femoris* in LBW swine fed a HF+Dairy diet. Dairy fat may play an important role as mentioned previously. Additionally, dairy products can also potentially increase the carcass lean yield. Pork carcass quality is mainly determined by carcass weight and carcass lean yield (Pomar and Marcoux, 2003). Increased dairy consumption may increase the carcass quality and potentially generate more economic value. It has also been suggested that the proportions of commercial primal cuts such as loin, belly or ribs have more market value than just total carcass weight and lean yield (Marcoux et al., 2007). From **Chapter 4** we learned that dairy intake increased proportions of pork loin, rib end and rear tender end and increased Type I muscle fiber diameter in *longissimus* (loin eye) muscle in LBW piglets. Essential amino acids such as leucine from dairy can potentially activate genes related to myogenesis such as mTOR and IGF-1 (Wan et al., 2016).

It might be challenging to convince the industry to consider applying dairy in the feed due to high cost. Therefore, we need to identify key components contributing to these beneficial effects and suggest potential adaptation to swine feed for more cost savings.

5.2.4 Low birth weight piglets in pork industry

In **Chapter 4**, we found no significant differences in postnatal growth and meat/carcass quality between weaning LBW and NBW piglets in the context of a HF diet. Our data was not entirely consistent with other studies on measuring these aspects in LBW swine (Gondret et al., 2006; Matyba et al., 2021, Li et al., 2017b; Liu et al., 2014). In the HF diet, we added lard as the main source of fat (with 47% of energy from fat), which is different from Gondret et al. (2006), Li et al. (2017b) and Matyba et al (2021). Despite similar dietary ingredients used in the HF diet, the swine breed and feeding periods are different between Liu et al. (2014) (Duroc x (Landrace x Yorkshire), 21 weeks) and our study (Landrace x (Large white x Duroc), 6 weeks). Additionally, piglets with LBW phenotype in our study ranged from 0.8 kg to 1.4 kg, compared with 0.8 kg to 1.1 kg in Gondret et al. (2006), 0.65±0.07 kg in Matyba et al. (2017), 0.95±0.05kg in Li et al. (2017b) and 0.95±0.03kg in Liu et al. (2014). Those piglets with stronger LBW phenotype might be more susceptible to developing metabolic syndrome and/or exhibiting pork quality demerits.

Therefore, it would be helpful to establish a standard such as validating a birth weight threshold to better identify piglets with increased risk on postnatal mortality and impaired growth, which can be in a breed-specific manner. We speculate that if a “benchmark” was established, piglets could be classified into different birth weight groups such as 1) severe LBW phenotype (unviable), 2) moderate LBW phenotype (viable but showing delayed growth), or 3) mild LBW phenotype (viable and showing normal growth). For instance, piglets found in group 3) may still be a viable market option due to the potential on catch-up growth and exhibiting

normal pork quality. Additionally, maternal nutrition during pregnancy and breeding techniques may be another approach to reduce the severity of LBW phenotype in their litters.

5.3 Limitations

To our knowledge, this is the first study to use a swine model of IR to investigate lipid incorporation of dairy-derived fatty acids in muscle tissues, as well as assessing the effect of dairy products on meat quality. Pigs have been known to have a greater capacity of pancreatic beta cells than rats and primates, which make them more resistant to develop diabetes compared with rats/mice (Denis et al., 2006). Our data reflects that LBW-HF group exhibited greater intra-group variations in terms of insulin sensitivity (i.e. fasting insulin and HOMA-IR). This could be a result of a short feeding period since longer feeding period (i.e. 3 to 12 months) may appear to be more consistent to fully induce IR (Hsu et al., 2017). In addition, sample sizes in both studies are also relatively small (n = 5~8). The power of our statistical comparisons was potentially limited due to small sample size and/or relatively short feeding period. Therefore, studies with longer intervention and bigger sample size could be more powerful to better understand the impact of diet and birth weight on metabolic complications (i.e. IR and dyslipidemia) and pork quality.

5.4 Future direction and conclusions

Infants with heavy birth weight (HBW) may also be at risk of childhood obesity, IR and hypertension (Kuciene et al., 2018; Wang et al., 2021). In this thesis, we used a 95% confidence interval (CI) to categorize LBW piglets (below 95% CI) or NBW piglets (within or above 95% CI). Those birth weight above 95% CI in our study could theoretically be identified as HBW piglets, and they were potentially at risk of IR. To be more specific, #32 and #33 from NBW-HF

group both had the heaviest birth weight of 2.0kg, which is greater than the NBW-HF group average of 1.73kg and beyond the 95% CI. Fasting insulin levels from these two piglets were 8.6 and 9.1 mU/L respectively, which is higher than the NBW-HF group average of 5.63 mU/L and is closer to the insulin-resistant (LBW-HF) group average of 9.9 mU/L. Therefore, it would be noteworthy for future studies to include this particular group to further investigate the impact of HBW and diet on glucose/insulin metabolism and cardiovascular risks.

Many studies have investigated the association between dairy intake and body composition (i.e. abdominal fat). Cohort studies have reported that daily consumption of 1~3 servings of dairy (specifically low-fat dairy) was associated with decreased visceral or subcutaneous fat in adolescents or middle/old-aged females (Moore et al., 2008; Murphy et al., 2020). It has also been suggested that increased dairy intake in the context of energy restriction can lead to a reduction in fat mass and body weight (Lopez-Sobaler et al., 2020). In this thesis, we found that the consumption of 3-servings of full-fat dairy decreased the subcutaneous fat depth in LBW piglets. Future studies could also investigate the impact of low/non-fat dairy on body fat composition in piglets. In addition, lean pork is also favored by both consumers and industry. Adding low/non-fat dairy might be more beneficial for producing leaner pork cuts compared with full-fat dairy, which could be investigated in future studies.

In summary, studies in this thesis provide new evidence on intestinal/muscle lipid metabolism and meat/carcass quality parameters. We have demonstrated that increased intestinal lipid absorption and preferential muscle lipid deposition in LBW swine can be explained by the upregulation of CD36. Increasing dairy intake can potentially add value to both pork (increasing long-chain PUFA content) and carcass (increasing the predicted lean yield) quality. Our findings

also suggest that LBW pork quality may not be necessarily impaired, which is dependent on LBW phenotype and feeding practice.

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APPENDICES

Appendix A. Health Benefits of Dairy Products on Insulin Resistance and Immunity

Appendix A is a mini-review paper that investigates beneficial effects of dairy products on human health and has been submitted to *Journal of Nutritional Science* (in review as of September 2022).

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1. Introduction

Obesity is a worldwide health issue with the prevalence rising dramatically in past decades. We know that obesity-related metabolic complications such as insulin resistance (IR) or type II diabetes (T2D) have been associated with an array of immune dysfunctions [(1)]. Chronic low-grade systemic inflammation has now been recognized by many as a key etiological factor for the development of obesity-related chronic disease such as cardiovascular diseases (CVD) and T2D. Dietary habits play an important role in the development of obesity, as well as relevant complications; this is also key in the treatment of such conditions [(2)]. More specifically, the acute effect of dairy products (i.e. milk, yogurt and cheese) on human health has been hotly debated regarding the improvement of insulin response, glycemic control or immune modulation. Yet, it is still relatively unclear why dairy products promote these beneficial effects. In this mini-review, we aim to present the evidence from observational studies or randomized controlled trials (RCT) that explore the relationship between dairy consumption and IR, T2D or immune function, as well as identifying the potential dairy-derived nutrients that may contribute to these health benefits.

2. Dairy consumption, insulin resistance and type II diabetes

IR occurs when cells in peripheral tissues do not respond to insulin properly with decreased glucose uptake, resulting in hyperglycemia. Pancreatic beta cells continue to produce and secrete more insulin in order to maintain the normal blood glucose level (euglycemia) until exhaustion or fatigue, eventually resulting in type II diabetes (T2D). It has been reported that the prevalence of IR has been as high as 45% in certain developing countries [3]. Dairy products have been shown to be positively associated with improvement in insulin sensitivity in either interventional or observational studies [4,5,6]. Daily dairy consumption has also been associated with a lower risk of insulin intolerance [4,5]. In addition, more frequent long-term consumption of dairy products (≥4 servings/day for 12 months) has been related to a decreased HOMA-IR score, indicating better insulin sensitivity [6]. Notably, there have been discrepancies in a number of public health guidance policies regarding the recommendation of dairy (and/or full fat dairy) intake in relation to health. For example, the latest Canada Food Guide in 2019 actually withdrew the recommendation on dairy consumption due to the concern on saturated fatty acids (SFA) [7]; the dietary guideline in the US also only recommends low-fat or fat-free milk or yogurt [8]. SFA has been linked to poor health outcomes, by increasing the risk of CVD and T2D. Yet, it is also noteworthy that not all SFA have the same atherogenic properties. For instance, ruminant-derived dairy products contain a diverse fatty acid profile, which consists of 8%~22% medium chain fatty acids (C6:0 to C12:0), 22%~35% palmitic acid (C16:0), 20%~30% oleic acid (C18:1n9), as well as polyunsaturated fatty acids (PUFA) and unique odd chain fatty acids [9]. Despite the deleterious effect of palmitic acid (C16:0) on insulin sensitivity, both butyric acid (C4:0) and myristic acid (C14:0) have shown a beneficial effect on glycemic control in mice [10]. Odd-chain fatty acids such as pentadecanoic acid (C15:0) and margaric acid (C17:0) were also associated with the lower risk of T2D [11]. In addition to dairy-derived fatty acids, another nutrient that can improve IR is the fat-soluble vitamin K2, which is synthesized by bacteria during the fermentation of cheese products [12]. The dietary intake of menaquinones (vitamin K2) was found to be associated with a reduced risk of T2D [13].

On the other hand, there have been an equal number of studies that found no positive association between dairy consumption and IR, leading to a controversy around the effect of dairy intake on insulin sensitivity [14,15]. Indeed, the form of dairy products included (i.e. low-fat, full-fat), the length of the intervention, as well as the number of participants may explain some of these discrepancies among studies. More clinical trials that carefully control for dairy intake and

that consider the food matrix when comparing low-fat to regular fat dairy are required to understand the relationship between dairy consumption and IR.

3. Dairy consumption and inflammations

Acute inflammation is considered a normal physiological response to infections with common symptoms of redness, heat, swelling, pain and loss of function. However, failure to regulate and resolve acute inflammation leads to tissue damage and metabolic dysfunction. Obesity is characterized as the state of chronic low-grade inflammation mainly due to the excessive secretion of pro-inflammatory cytokines from adipose tissue that contribute to the progression of IR and dyslipidemia [16]. Previous findings from meta-analyses and systematic reviews reported that dairy consumption, irrespective of their fat content, exert neutral effects on inflammation in humans [14,15]. A recent RCT conducted by Schmidt et al. revealed that daily intake of 3.3 servings of either low-fat or high-fat dairy products (specifically milk, yogurt and cheese) did not modulate circulating C-reactive protein, interleukin-6 (IL-6) nor adiponectin concentrations compared to limited dairy intake in 72 participants [17]. In addition to the beneficial effect on lowering the risk of T2D, the unique odd-chain fatty acid derived from dairy fat, specifically C15:0 has been reported to exert anti-inflammatory properties [11]. Oral supplementation of C15:0 to obese mice fed a high-fat diet was found to significantly attenuate monocyte chemoattractant protein 1 (MCP-1) and IL-6 concentrations compared to un-supplemented groups [11].

In contrast, we also acknowledge that people are in the non-fasting (postprandial) state for most of the day. The established link between postprandial inflammation and CVD has led to extensive investigation on understanding how nutrients can modulate the inflammatory response specifically during the postprandial state. We know that levels of neutrophils, lymphocytes and total leukocytes can become elevated acutely (1-6 hrs) in response to a fat meal [18]. Evidence also suggests that high fat meals are associated with increased endotoxin levels (also known as lipopolysaccharides, LPS), which may further increase acute postprandial inflammation [19]. Pei et al. demonstrated that premeal low-fat yogurt consumption attenuated postprandial plasma IL-6 concentrations and LPS binding protein to sCD14 ratio in both obese and non-obese subjects after a high-fat high-carbohydrate meal [20]. Similarly, another randomized crossover study

reported that both probiotic yogurt and acidified milk intake significantly reduced postprandial IL-6, tumor necrosis factor-alpha (TNF-a) and chemokine ligand 5 concentrations in response to a high-fat meal challenge in fourteen healthy men [21]. Collectively, these findings suggest that fermented dairy products may exert promising benefits in counteracting the postprandial inflammatory response to high-fat meal consumption. Although the underlying mechanisms are not fully understood, it is likely due to a combination of favorable alteration(s) of gut microbiota and intestinal permeability associated with fermented dairy intake.

4. The potential benefits of dairy intake on immune function

The immune system consists of two major networks that work closely together to mount an appropriate response to foreign stimuli, namely innate immunity and adaptive immunity. Innate immune system consists of physical defense barriers as well as innate immune cells, whereas the latter consists of T and B lymphocytes. Currently, the understanding on dairy intake and immune function remains unclear. However, previous studies from our group have demonstrated that dietary intake of vaccenic acid (*trans* C18:1) (a natural trans fatty acid commonly found in dairy products), can potentially improve immune function [22]. We also know that dairy products are a major source of dietary choline [23]. Polar lipids (in the form of phosphatidylcholine (PC) and sphingomyelin (SM)), are naturally present in the milk fat globular membrane. We note that the consumption of lipid soluble forms of choline has been shown to have a promising ability to support both immune system development and function in Sprague-Dawley rats [24,25]. However, low-fat dairy also appears to be a rich source of choline; perhaps more so than high-fat dairy [26]. Preliminary findings from our group have shown that consumption of 3 servings per day of low-fat dairy improved T cell function in a swine model of IR [27]. These data provide novel insights to support the role of dairy intake in modulating immune function.

5. Conclusions

The food matrix includes a unique combination of nutrients that can elicit additive and synergistic interactions, which is particularly evident with nutrient dense food groups such as dairy. The effect of dairy products on IR and immune function remains complex. Studies have

shown a potential beneficial effect of dairy consumption on the improvement of IR and the modulation of immune function partially due to dairy-derived odd-chain fatty acids and choline. Despite these advances, more clinical trials are required to validate and translate these findings.

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Appendix B. Effect of high-fat and low-fat dairy products on cardiometabolic risk factors and immune function in a low birthweight swine model of diet-induced insulin resistance

Appendix B is a research paper that compares low-fat vs high-fat dairy products for their effects on cardiometabolic health and immune function in this swine model of IR. The paper has been published in *Frontiers in Nutrition* (doi: 10.3389/fnut.2022.923120).

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Introduction

The immune system consists of two major arms, namely innate (e.g., skin, mucosal, myeloid immune cells) and adaptive (T and B lymphocytes) immunity, that together mount an appropriate immune response to foreign stimuli. Proper immune response to infections is influenced by genetics, age, sex, smoking, alcohol, as well as the presence of obesity and nutrition (Calder, 2020; Zimmermann & Curtis, 2019). In the context of obesity, alterations in the secretory output from adipose tissue is characterized by an increased release of cytokines and chemokines resulting in an excessive recruitment, infiltration and polarization of macrophages towards a pro-inflammatory phenotype (i.e. M1-like macrophages) (Zatterale et al., 2020). On the other hand,

obesity-related metabolic complications are also associated with an increased intestinal permeability which further promotes systemic inflammation through the translocation of endotoxin into circulation (Nagpal et al., 2018). Obesity-induced inflammation is also known to impair insulin signaling which contributes to insulin resistance and dyslipidemia and ultimately to the progression of cardiovascular disease (CVD) (Powell-Wiley et al., 2021a). We have previously demonstrated that individuals with obesity and type 2 diabetes (T2D) have an impaired T cell and neutrophil response following mitogen stimulation (Richard et al., 2017) compared to metabolically healthy individuals with obesity. This suggests that regardless of the obesity status, hyperglycemia and/or insulin resistance further impairs immune function. More recently, obesity and T2D have also been identified as major risk factors for severe COVID-19 outcomes (Sjögren et al., 2021) contributing to the body of evidence demonstrating that obesity impairs immune function.

Apart from genetic, lifestyle and environmental factors, nutrients play critical roles in modulating obesity-related cardiometabolic perturbations and immune dysfunction. Fatty acids are key components in modulating the blood lipid profile, while also exerting pro/anti-inflammatory activities (M. L. Fernandez & West, 2005; Fritsche, 2015). In that regard, saturated fatty acids (SFA) are generally considered pro-inflammatory, yet not all SFA exert the same pro-inflammatory properties (Fritsche, 2015). Medium-chain SFA may be beneficial for weight loss and major CVD risk factors (Bohl et al., 2017; Kris-Etherton & Fleming, 2015). Pentadecanoic acid (C15:0), the ruminant-derived odd-chain SFA, has been shown to exert anti-inflammatory activity (Venn-Watson et al., 2020) and be inversely associated with variables of insulin resistance and T2D (Imamura et al., 2018a). Additionally, the natural *trans*-fat vaccenic acid (18:1 *trans*-11) has been found to be beneficial to immune function (Blewett et al., 2009). Recent

work from our group have also demonstrated the promising role of dietary choline, particularly in the forms of phosphatidylcholine (PC) and sphingomyelin (SM), at improving the immune system development and function in rats (Azarcoya-Barrera et al., 2020, 2021a). Dairy products are unique and possess a complex food matrix that contains significant amounts of choline and medium-chain SFA along with other important nutrients. Still, many consumers are avoiding dairy products due to the perception that diets high in SFA may lead to adverse effects on health. In contrast, emerging evidence now point towards a neutral and potentially beneficial effect of total dairy and dairy fat intake on most CVD risk factors including blood pressure, inflammation, T2D and dyslipidemia (J. P. Drouin-Chartier, Brassard, et al., 2016; J. P. Drouin-Chartier, Côté, et al., 2016; J. P. Drouin-Chartier et al., 2021; Hirahatake et al., 2020; Liang et al., 2018).

Yet, there is ongoing debate on the contribution of dairy fat *per se* on overall cardiometabolic health. One of the major reasons for this is that most studies to date have not considered the complex food matrix when comparing high-fat vs. low-fat dairy products (i.e., comparing milk to cheese or milk to butter) (Brassard et al., 2017; Rancourt-Bouchard et al., 2020). There are very limited studies that have assessed the effect of dairy foods and dairy fat, considering the importance of the food matrix, on immune function. Considering the glucose, insulin and immune modulatory effects attributed to certain nutrients found in dairy foods and dairy fat, the overall aim of this study was to determine the effect of consuming 3 servings/day of high-fat vs. low-fat products on obesity-related cardiometabolic perturbations and immune function in a pre-established swine model of insulin resistance. Commercially available high-fat and low-fat dairy products of similar food matrix were chosen. We hypothesized that consumption of 3 servings/day of dairy products, irrespective of the fat content, would exert a neutral effect on the lipid profile. We also proposed that feeding high-fat dairy may provide greater benefits on

glucose metabolism, inflammation and immune function due to the presence of unique bioactive nutrients in dairy fat.

Materials and Methods

Animals and housing

All piglets were born and raised at Swine Research Technology Centre (SRTC), Department of Agricultural, Food and Nutritional Science, University of Alberta, Canada. Piglets used in the present study are offspring of a cross of Duroc boar and Large White-Landrace sow. Only male piglets were selected due to the susceptibility of early insulin resistance. The selection criteria were previously described (Fontaine et al., 2019a). Briefly, a mean litter weight and standard deviation was determined to find a 95% confidence interval (CI), categorizing piglets as low birthweight (LBW, less than the 95% CI) or normal birthweight (NBW, within or above the 95% CI). A t test was used to ensure a significant difference in birth weight between the 2 groups. Water, temperature control and routine health checks were all done daily by trained staff at the SRTC. Piglets were socialized regularly by trained staff to minimize stress response during subsequent experimental procedures. All study protocols were approved by University of Alberta Animal Care and Use Committee and in accordance with regulations of Canadian Council of Animal Care (Protocol AUP00001184).

Study design

All piglets were fed with SRTC pig grower diet (Chow) after weaning and until 5 weeks of age. At 5 weeks of age, LBW piglets were randomized to consume one of the 3 experimental diets: 1)

control high-fat (CHF), 2) CHF supplemented with 3 servings per day of high-fat dairy products (HFDairy) or 3) CHF supplemented with 3 servings per day of low-fat dairy products (LFDairy). As comparison groups, NBW piglets were fed a Chow or CHF diet. A total of 35 pigs (LBW-CHF n=8, LBW-HFDairy n=8, LBW-LFDairy n=8, NBW-CHF n=6, NBW-Chow n=5) completed the study and were fed for a total of 7 weeks, including 1 week of CHF transition diet (**Figure 1**). At 12 weeks of age, pigs were fasted overnight, and fasting blood were collected and then euthanized by trained staff at the SRTC.

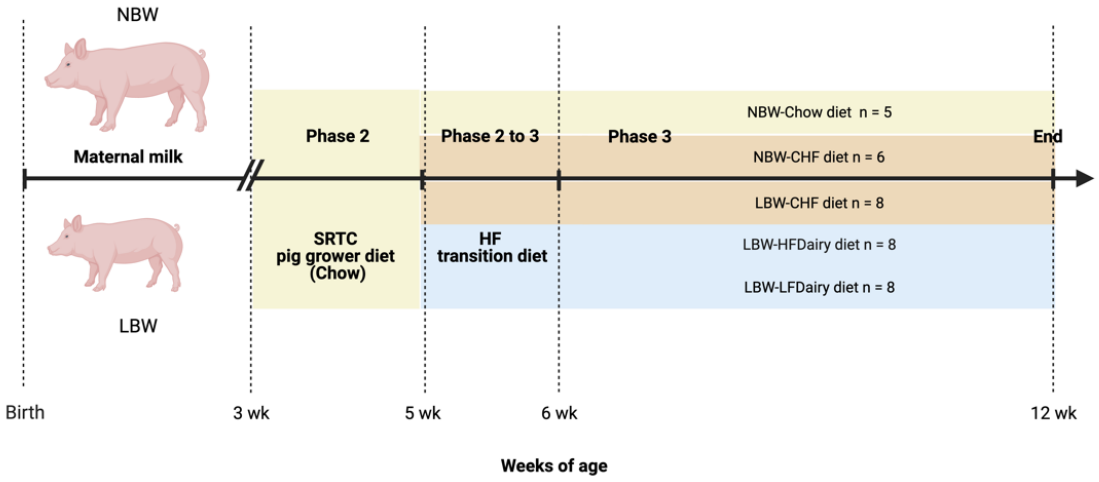

Figure 1. Study design to investigate the effects of consuming dairy fats on cardiometabolic risks and immune function in swine model of IR (figure created with BioRender)

CHF, control high-fat diet; HF, high-fat; HFDairy, high-fat dairy diet; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight; SRTC, swine research technology centre.

Diet

The detailed nutrient composition of the experimental CHF diet has been previously described (Fontaine et al., 2019a; Singh et al., 2021). Briefly, the respective percentages of energy (Kcal) derived from fats, carbohydrates and protein were 46% (mainly lard), 33% (with 17% calories from fructose specifically) and 21%. 1% w/w cholesterol was also added. In contrast, the Chow diet consisted of 14% total energy from fat, 69% total energy from carbohydrate and 17% total energy from protein. To understand the effect of dairy products and more specifically dairy fat, the CHF diet was supplemented with 3 servings/2000 Kcal per day of either low- or high-fat dairy products. In the LFDairy diet, one serving of skimmed milk powder (0% fat; No Name®, Canada), plain yogurt (1% fat; Foremost Farms, Canada) and mozzarella cheese (18% fat; No Name®, Canada) was fed to each pig daily contributing to approximatively 16% of total energy intake in that group (**Table 1**). In the HFDairy diet, one serving of whole milk powder (3.25% fat; Bulk Barn, Canada), plain yogurt (10% fat; Liberté, Canada) and mozzarella cheese (28% fat; No Name®, Canada) was fed to each pig daily contributing to approximatively 28% of total energy intake in that group. The serving size for each dairy product was based on the previous Canada's food guide (version 2007) which consisted of 250 mL of milk or powdered milk (24 g low-fat milk powder, 33.5 g high-fat milk powder), 175 mL of yogurt and 50 g of cheese regardless of their fat content. The number of servings provided per day was adjusted weekly proportionally to their increasing energy intake to maintain a constant ratio of 3 servings per 2000 Kcal intake. All products were available and purchased *via* retail outlets fresh every 10 days. Since pigs in both dairy groups did not lower their consumption of the experimental CHF diet, these two groups were pair-fed to the group receiving the experimental CHF diet only. Therefore, less experimental CHF diet was added to the feeder to compensate for the amount of calories coming from dairy products so that energy intake would be consistent across all three

LBW groups. Body weight was measured weekly while food intake was measured and adjusted daily.

Table 1. Nutrient composition of dairy products per 2000 Kcal diet

	Amount per serving (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Milk Powder, 3.25% MF	33	174.9	8.91	8.91	13.2
Greek Yogurt, 10% MF	175	200	6	17.5	5
Mozzarella Cheese, 28% MF	50	183.3	11.7	13.3	1.7
Milk Powder, 0% MF	24	90	9	0.18	12.6
Greek Yogurt, 1% MF	175	100	8	1.5	14
Mozzarella Cheese, 18% MF	50	150	15	8.3	1.7

Nutrient composition was calculated based on serving size of Canada's Food Guide (2007) in a 2000 Kcal diet. All dairy products were purchased from local distributor. MF, milk fat; Greek yogurt (1% MF), Foremost Farms, Canada; Greek yogurt (10% MF), Liberté, Canada; Milk powder (0% MF), No Name®, Canada; Milk powder (3.25% MF), Bulk Barn, Canada; Mozzarella cheese (18% MF); No Name®, Canada; Mozzarella cheese (28% MF), No Name®, Canada.

Tissues and blood processing and peripheral blood mononuclear cells (PBMCs) isolation

Fasting blood samples were collected in tubes containing EDTA, dipeptidyl peptidase 4 inhibitor (EMD Millipore, MA), and Complete® general protease inhibitor (Sigma-Aldrich, USA) before being centrifuged at 3000 rpm for 10 min to obtain plasma. Plasma was aliquoted and stored at -80°C until further analysis. To isolate PBMCs, 1% BSA (bovine serum albumin) in PBS (phosphate-buffered saline) was added to dilute and resuspend the buffy coat. Cell suspension

was then layered over 5 mL histopaque and followed by centrifugation at 1800 rpm for 30 minutes. PBMCs were recovered from the gradient interface, washed with 1% BSA in PBS, and frozen in 10% complete culture medium (RPMI 1640 media; Life Technologies, Burlington, ON, Canada) with 10% DMSO at -80°C before being transferred to liquid nitrogen. Samples of liver were collected and treated with ice-cold saline solution and snap frozen in liquid nitrogen prior to be stored at -80°C until further processing.

Plasma biomedical and liver fatty acid analysis

Fasting plasma concentrations of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and glucose were assessed using commercial enzymatic colorimetric kits (Wako Pure Chemicals, Tokyo, Japan) and described previously (Fontaine et al., 2019a; Singh et al., 2021). Plasma insulin concentration were measured using commercial porcine-specific ELISA kits as per manufacturer's instructions (Mercodia, USA). Additional aliquots of plasma within the same group were mixed and measured for lipoprotein associated plasma TG and cholesterol fractions by fast protein liquid chromatography (FPLC) at the University of Alberta lipidomic core facility as described elsewhere (H. Wang et al., 2007). Fasting plasma inflammatory marker concentrations were assessed by porcine cytokine/chemokine 13-Plex Discovery Assay® Array at Eve Technologies (Calgary, Canada).

Liver tissue fatty acid profile was assessed by gas chromatography equipped with a flame ionization detector (Agilent 8890). Briefly, liver tissue samples were pre-treated with tissue homogenizer, and only the homogenates were collected for analysis. 200 µL homogenate was pipetted into large glass tube and followed by conventional Folch extraction for total lipids. After

sitting overnight at 4°C, the bottom layer of the lipid phase was dried down under nitrogen gas. 1.5 mL of methanolic KOH was then added followed by saponification at 110°C for 1 hour. Methylation of fatty acids was conducted by adding 1.5 mL BF3 (boron trifluoride) and 1.5 mL hexane followed by incubating at 110°C for 1 hour. After cooling down,1mL of deionized water was added. The top layer was then collected, dried down, reconstituted with hexane and then stored at -80°C until further analysis. All fatty acids in liver were expressed as percentage of total identified fatty acids.

PBMC membrane phospholipid analysis

Phospholipids in PBMCs membrane were analyzed by high performance liquid chromatography (HPLC) at University of Alberta Lipidomic Core Facility. Briefly, isolated cells were resuspended in 200 μL PBS and sonicated to homogenize and disrupt cell membranes. Cell homogenate was then measured for protein content by standard BCA (bicinchoninic acid) assay and followed by Folch extraction. Internal standards were added to quantify phospholipids. After sitting overnight at 4°C, the bottom layer of the lipid phase was dried down under nitrogen gas and redissolved with chloroform:isooctane (1:1) and transferred to HPLC vial for further analysis. The proportion of PC and phosphatidylethanolamine (PE) was determined, and ratio was calculated.

PBMCs phenotype analysis

Two to four multicolor flow cytometry panels were designed and PBMCs were stained with different antibodies as follow: T cell panel: CD3 (PerCP), CD4 (FITC), CD4 (PerCP), CD8 (PE), CD25 (STAR PE), CD80 (APC), and CD45RA (PE); Antigen-presenting cell (APC) panel: CD14 (FITC), CD11c (PE), CD284 (AF647), SLAII (FITC) and CD21 (PE). Briefly, 100 μL of

whole blood was added to pre-treated 96 well plates and incubated twice with lysis buffer. Lysed cells were then washed with 5% FCS (fetal calf serum) in PBS and spun. Cells were then treated with 2 to 4-color flow cytometry recognized fluorescence-conjugated antibodies (all purchased from BD Biosciences, BioLegend, Bio-Rad Laborarories, USA). After incubation at 4°C for 30 minutes, cells were washed with PBS and fixed in paraformaldehyde (10 g/L, Thermo Fisher Scientific) until further analysis. All samples were acquired within 72 hours of preparation by BD LSRFortessa X20 SORP flow cytometer at University of Alberta and data was analyzed using FlowJo v10 (USA).

Ex vivo cytokine production from mitogen stimulated PBMCs

Isolated PBMCs (1.25 x 10^6 cells/mL) were cultured without mitogens (unstimulated) or with mitogens at corresponded concentration: 1) phorbol myristate acetate-ionomycin (PMA-I, T cell mitogen, Fisher Scientific, 2 µl/mL), 2) phytohemagglutinin (PHA, T cell mitogen, Sigma-Aldrich, 5 mg/mL), 3) pokeweed mitogen (PWM, T and antigen presenting cells mitogen, Sigma-Aldrich, 55 µl/mL). After 48 hours incubation, samples were spun at 1500 rpm for 10 minutes to pellet cells. Supernatant was collected and stored at -80°C. Concentrations of interleukin (IL)-1β, IL-2, IL-6, IL-10, IFN-γ and TNF-α were measured by commercial porcine specific ELISA kits (R&D systems, Minnesota) following manufacturer's instructions. Concentrations of cytokines produced from PBMCs were quantified by using a microplate reader (SpectraMax 190, Molecular Devices). Samples were assayed in duplicate with CV<10%.

Statistical analyses

All statistics were performed by using one-way ANOVA procedure (Prism 8) with Tukey adjustment for multiple comparisons between groups. All tests and comparisons were considered

significant at P value < 0.05. Based on previous studies using our diet induced swine model of insulin resistance, we have been able to detect 20% differences on average with a 95% CI with n = 4-5 piglets per group when comparing phenotype differences (15-20% difference; LBW *versus* NBW) and diet induced differences of fasting lipid profile, insulin and glucose (20-30% difference; CHF *vs* Chow). As this was our first time assessing immune function in this pre-established model, we aimed to increase our sample size to n = 8 piglets per group in order to detect significant differences in immune outcomes. All results were expressed as means ± standard error mean (SEM) unless otherwise stated.

Results

Pig growth and daily food intake parameters

As expected, the birthweights of piglets in all three LBW groups were lower than piglets in both NBW groups (all, P < 0.05, **Supplementary Table 1**). The NBW-Chow group had a lower average of energy intake compared to all high-fat diet fed groups (all, P < 0.05). The energy to weight gain ratio of NBW-Chow piglets were also lower than all other groups (all, P < 0.001); however, there were no differences in average daily growth and final bodyweight in all groups.

Supplementary Table 1. Growth and feed consumption of LBW and NBW swine fed different experimental diets

	NBW-Chow	NBW-CHF	LBW-CHF	LBW-HFDairy	LBW-LFDairy	*P*
Birthweight (kg)	1.62±0.07[a]	1.73±0.10[a]	1.29±0.08[b]	1.23±0.06[b]	1.23±0.09[b]	<0.001
Final body weight (kg)	48.44±3.16	53.62±1.97	48.10±2.77	50.53±2.81	49.49±1.88	0.618
Average daily growth (g/day)	779.20±47.34	854.00±15.80	794.70±41.00	824.10±40.47	815.40±27.78	0.723
Average energy intake (Kcal/day)	4077±290.10[a]	5717±340.30[b]	5314±237.90[b]	5716±250.60[b]	5678±172.20[b]	0.001
Energy to gain ratio	5.23±0.14[a]	6.67±0.29[b]	6.72±0.15[b]	6.96±0.14[b]	6.97±0.10[b]	<0.001

CHF, control high-fat diet; HFDairy, high-fat dairy diet; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

Fatty acid profile in liver tissue

The analysis was conducted in all LBW groups only, as CHF is considered the control group for comparing the effect of dairy in this model and are presented in **Supplementary Table 2**. In liver tissue, the proportion of myristic acid (C14:0) was higher in LBW-HFDairy compared to both LBW-CHF and LBW-LFDairy (both $P = 0.001$). Similarly, the unique dairy-derived odd-

chain fatty acid, pentadecanoic acid (C15:0), was also higher in LBW-HFDairy compared to both LBW-CHF and LBW-LFDairy (both $P < 0.004$). The proportion of eicosapentaenoic acid (EPA, C20:5 n3) was higher in LBW-CHF compared to LBW-HFDairy only ($P < 0.01$). There were no significant differences in the proportion of total SFA, mono- (MUFA) and polyunsaturated fatty acids (PUFA) across all LBW groups.

Supplementary Table 2. Liver total fatty acids profile in LBW swine fed experimental diets at 12 weeks of age

	LBW-CHF	LBW-LFDairy	LBW-HFDairy	*P*
Total SFA	45.60±0.61	45.86±0.41	47.02±0.38	0.565
Total MUFA	17.35±0.60	17.30±0.93	16.63±0.70	0.795
Total PUFA	36.47±0.40	36.23±0.61	35.72±0.55	0.907
C14:0	0.31±0.02[b]	0.37±0.03[b]	0.56±0.04[a]	<0.001
C15:0	0.24±0.02[b]	0.25±0.01[b]	0.32±0.01[a]	<0.001
C16:0	15.12±0.43	15.63±0.46	16.57±0.68	0.179
C16:1	0.66±0.05	0.69±0.06	0.63±0.04	0.744
C17:0	0.58±0.03	0.61±0.03	0.62±0.05	0.756
C18:0	29.23±0.70	28.95±0.79	28.71±0.90	0.902
C18:1	16.29±0.56	16.21±0.88	15.67±0.66	0.801
C18:2n6	15.41±0.47	15.30±0.36	14.88±0.27	0.581
C18:3n6	0.17±0.03	0.15±0.01	0.17±0.02	0.701
C18:3n3	0.19±0.01	0.17±0.01	0.17±0.01	0.249
C20:0	0.46±0.06	0.41±0.03	0.41±0.03	0.581
C20:1	0.23±0.01[a,b]	0.23±0.01[a]	0.19±0.01[b]	0.018

C20:2n6	0.48±0.01[a]	0.49±0.01[a]	0.41±0.02[b]	<0.001
C20:3n6	1.57±0.11	1.86±0.09	1.52±0.12	0.072
C20:4n6	10.08±0.29	9.98±0.48	10.69±0.35	0.373
C20:5n3	2.25±0.12[a]	1.83±0.10[a,b]	1.51±0.22[b]	0.011
C24:0	0.24±0.01	0.24±0.02	0.46±0.20	0.336
C24:1	0.17±0.01	0.17±0.01	0.14±0.01	0.116
C22:5n3	1.28±0.07	1.31±0.10	1.37±0.07	0.711
C22:6n3	5.05±0.34	5.15±0.43	5.01±0.37	0.965

CHF, control high-fat diet; HFDairy, high-fat dairy diet; LBW, low birthweight; LFDairy, low-fat dairy diet; MUFA, monounsaturated fatty acid; NBW, normal birthweight; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.All values are expressed as means ±SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

Fasting plasma lipids, glucose, insulin, HOMA-IR and inflammatory marker profiles

Fasting plasma lipid profile, glucose, insulin and calculated HOMA-IR are shown in **Table 2**. Fasting glucose in NBW-Chow was lower than in NBW-CHF and LBW-CHF (both $P < 0.01$). The LBW-HFDairy had lower plasma glucose concentrations compared to the LBW-CHF group ($P < 0.05$). Feeding low-fat dairy products also improved (lowered) fasting glucose concentrations in that they were no longer different to NBW-Chow, but to a lesser extent than high-fat dairy products. Although no statistical differences were observed in fasting insulin concentrations and HOMA-IR among all groups, there was a trend toward higher HOMA-IR in the LBW-CHF group when compared to all other groups (P-model = 0.08) suggesting early signs of insulin resistance.

Table 2. Fasting plasma lipid, glucose and insulin concentrations in LBW and NBW swine fed different experimental diets

	NBW-Chow	NBW-CHF	LBW-CHF	LBW-HFDairy	LBW-LFDairy	*P*
Glucose, mmol/L	4.44±0.31[c]	5.99±0.29[a,b]	6.27±0.37[a]	5.05±0.27[b,c]	5.50±0.15[a,b,c]	0.001
Insulin, mIU/L	3.66±0.36	5.63±0.88	8.83±2.97	3.67±0.48	3.72±0.51	0.094
HOMA-IR	0.72±0.07	1.52±0.27	2.69±1.06	0.82±0.12	0.92±0.13	0.08
TG, mg/dL	24.26±2.59	25.24±4.92	29.43±2.07	40.15±8.72	27.34±1.86	0.156
TC, mg/dL	92.32±4.42[a]	300.20±25.36[b]	252.60±17.36[b]	260.60±27.63[b]	275.30±25.64[b]	<0.001
LDL-C, mg/dL	54.08±2.03[a]	177.40±14.48[b]	158.30±15.38[b]	160.30±18.75[b]	167.40±18.72[b]	<0.001
HDL-C, mg/dL	24.44±3.10	26.10±3.54	24.06±2.81	20.10±1.06	22.71±1.74	0.572

CHF, control high-fat diet; HDL-C, high-density lipoprotein cholesterol; HFDairy, high-fat dairy diet; HOMA-IR, homeostatic model assessment for insulin resistance; LBW, low birthweight; LDL-C, low-density lipoprotein cholesterol; LFDairy, low-fat dairy diet; NBW, normal birthweight; TC, total cholesterol; TG, triglycerides. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

There were no differences in plasma TG and HDL-C concentrations across all groups. However, plasma TC concentrations in NBW-Chow were lower than in all other high-fat diet fed groups (all, $P < 0.001$). Similarly, LDL-C concentrations in NBW-Chow were lower compared to all

other groups (all, $P < 0.05$). Consistent with this, FPLC results showed that the NBW-Chow had the lowest amount of cholesterol in very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL particles compared to all other high-fat diet fed groups (**Figure 2**). Although no significant difference was observed in fasting plasma TG levels among groups, the three LBW groups had increased TG in VLDL particles compared to both NBW groups.

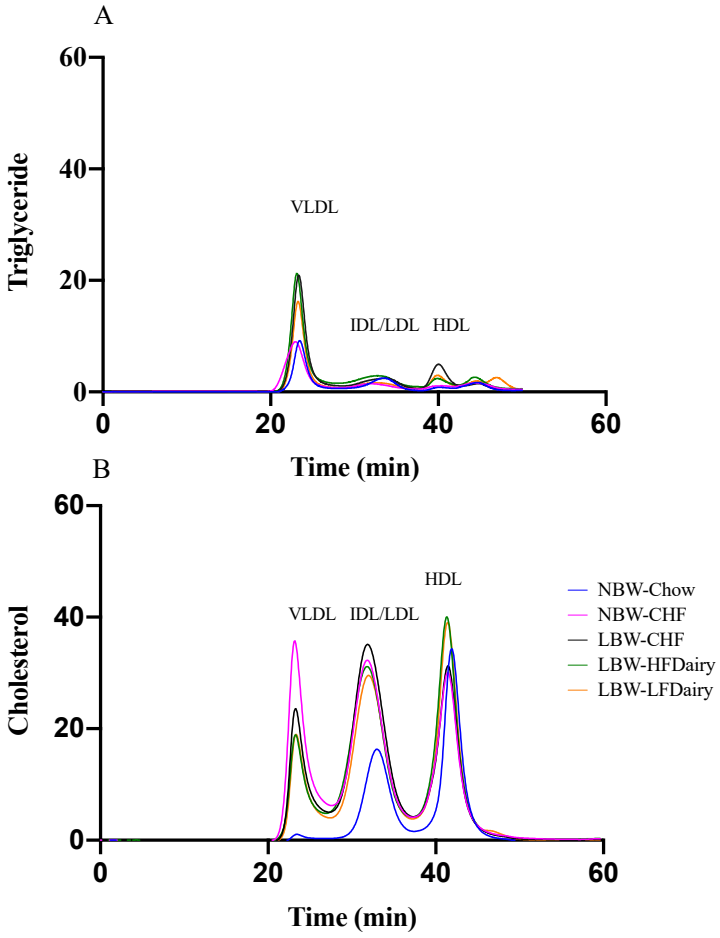

Figure 2. Fasting plasma cholesterol (A) and triglyceride (B) in lipoprotein subfractions measured by FPLC

CHF, control high-fat diet; FPLC, fast protein liquid chromatography; HDL, high-density lipoprotein; HFDairy, high-fat dairy diet; IDL, intermediate-density lipoprotein; LBW, low birthweight; LDL, low-density lipoprotein; LFDairy, low-fat dairy diet; NBW, normal birthweight; VLDL, very low-density lipoprotein

Fasting circulating inflammatory marker concentrations are shown in **Table 3**. IL-1α and IL-1β in LBW-HFDairy were higher than in NBW-CHF (both $P < 0.05$). IL-4 in LBW-HFDairy was higher than in both NBW-Chow and NBW-CHF (both $P < 0.05$). There were no differences in IL-1Ra, IL-2, IL-6, IL-8, IL-10, IL-12. IL-18, IFN-γ and TNF-α concentrations across all the groups.

Table 3. Fasting plasma inflammatory markers in LBW and NBW swine fed different experimental diets

	NBW-Chow	NBW-CHF	LBW-CHF	LBW-HFDairy	LBW-LFDairy	*P*
IL-1α, pg/mL	9.7±2.6[a,b]	10.1±1.4[b]	16.3±7.3[a,b]	36.1±7.9[a]	16.6±4.8[a,b]	0.029
IL-1β, pg/mL	80.8±15.0[a,b]	74.7±9.7[b]	186.7±62.4[a,b]	310.4±77.2[a]	128.4±35.5[a,b]	0.031
IL-1Ra, pg/mL	207.5±56.3	200.6±25.6	307.6±34.8	378.5±47.9	314.0±48.9	0.051
IL-2, pg/mL	56.4±12.9	61.9±14.2	122.7±53.9	224.7±57.4	107.4±36.6	0.087
IL-4, pg/mL	140.2±48.9[b]	175.3±23.8[b]	312.3±169.1[a,b]	914.9±227.3[a]	456.4±137.0[a,b]	0.013
IL-6, pg/mL	34.9±7.2	31.0±4.9	60.7±26.5	103.8±27.7	46.7±13.7	0.135
IL-8, pg/mL	52.8±2.8	55.0±3.1	61.2±6.9	58.4±3.6	55.1±1.1	0.682
IL-10, pg/mL	160.9±35.4	149.7±39.1	356.8±155.7	604.5±173.7	392.9±124.2	0.157
IL-12,	575.7±156.8	669.3±72.8	757.3±134.9	666.8±87.9	519.3±61.7	0.548

pg/mL						
IL-18, pg/mL	628.0±73.0	582.3±94.2	1039±295.8	1472±310.7	866.7±220.2	0.110
TNF-α, pg/mL	59.7±12.5	76.6±6.6	72.7±8.9	90.6±16.2	80.6±10.7	0.518
IFN-γ, pg/mL	4290±1022	3893±429	3469±375.9	3421±368.7	2920±93.5	0.423
IL4/IFN-γ ratio	0.03±0.01[b]	0.05±0.01[b]	0.08±0.04[b]	0.30±0.06[a]	0.14±0.05[a,b]	0.001

CHF, control high-fat diet; HFDairy, high-fat dairy diet; IFN-γ, interferon-gamma; IL, interleukin; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight; TNF-α, tumor necrosis factor-alpha. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

PBMC membrane phospholipid classes

No differences were observed in the total amount of the different phospholipid classes in PBMCs membrane except for PE and PC. PE and PC were found to be higher and lower, respectively, in the NBW-Chow and LBW-LFDairy groups compared to the LBW-CHF group (both $P < 0.05$, data not shown). In **Figure 3**, the ratio of PC:PE was found to be lower in NBW-Chow and LFDairy groups compared with both the NBW-CHF and LBW-CHF groups ($P < 0.001$).

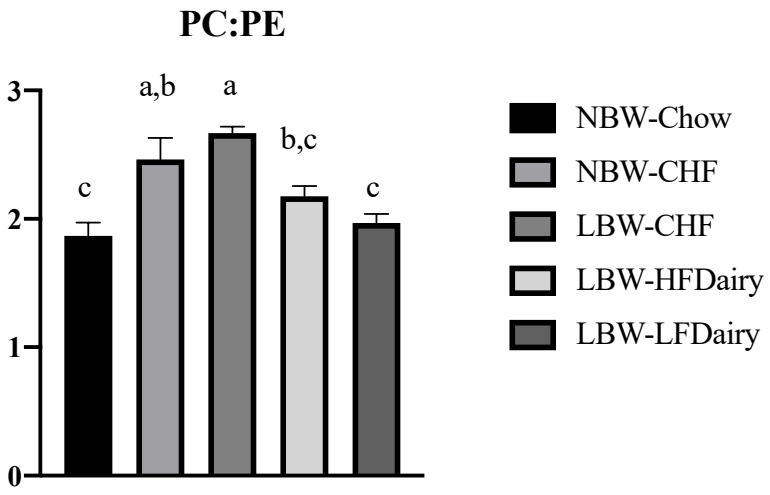

Figure 3. The phosphatidylcholine to phosphatidylethanolamine ratio in PBMC membrane CHF, control high-fat diet; HFDairy, high-fat dairy diet; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight; PBMC, peripheral blood mononuclear cell; PC, phosphatidylcholine; PE, phosphatidylethanolamine. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

Ex vivo cytokine production from mitogen stimulated PBMCs

Ex vivo cytokine production by PBMCs stimulated with mitogens is presented in **Table 4**. Following PWM stimulation, IL-2, TNF-α and IFN-γ production were significantly lower in LBW-CHF than in NBW-Chow (all, $P < 0.001$). While feeding high-fat dairy products had minimal effect on T helper cell (Th) 1 cytokine production, feeding low-fat dairy products significantly improved IL-2, TNF-α and IFN-γ production compared to LBW-CHF (all, $P < 0.05$). Additionally, LBW-CHF piglets were also found to produce less IL-1β compared to NBW-Chow ($P < 0.01$) and less IL-10 compared to both NBW-Chow ($P < 0.001$) and NBW-CHF ($P < 0.001$). Dairy products normalized the IL-1β production in that it was no longer different from the NBW-Chow group but not IL-10 production.

Following PHA stimulation, IL-2 production was significantly lower in LBW-CHF than in NBW-Chow and NBW-CHF (all, $P < 0.05$). While Feeding high-fat dairy products had minimal effects on IL-2 production, feeding low-fat dairy products significantly improved IL-2 production compared to LBW-CHF ($P < 0.01$). TNF-α and IFN-γ production also tended to be lower in LBW-CHF than in NBW-Chow and NBW-CHF (all, P model < 0.05). Similarly, while feeding high-fat dairy products had minimal effects on TNF-α and IFN-γ production, feeding low-fat dairy products significantly improved IFN-γ production compared to LBW-CHF ($P < 0.05$). Additionally, LBW-CHF was found to produce less IL-10 compared to NBW-Chow ($P < 0.01$); however, dairy products had minimal effect.

Following PMA-I stimulation, TNF-α production tended to be lower in LBW-CHF than in NBW-Chow and NBW-CHF (P model < 0.05). While feeding high-fat dairy products had minimal effect on TNF-α production, feeding low-fat dairy products significantly improved TNF-α production compared to LBW-CHF ($P < 0.01$). IL-6 and IL-10 production were also lower in LBW-CHF than in NBW-Chow (all, $P < 0.05$), and the production of IL-10 was no longer different compared to NBW-Chow in both LBW-HFDairy and LBW-LFDairy groups.

Table 4. *Ex vivo* cytokine production by mitogen stimulated PBMCs in LBW and NBW swine fed different experimental diets

	NBW-Chow	NBW-CHF	LBW-CHF	LBW-HFDairy	LBW-LFDairy	*P*
PWM (T and APC cells mitogen)						
IL-2, pg/mL	494.7±76.6[a]	297.0±39.8[b,c]	130.7±26.0[c]	148.9±16.1[c]	313.8±55.5[a,b]	<0.001
TNF-α, pg/mL	1385±195.5[a]	567.8±57.8[c]	686.3±82.2[c]	691.3±56.1[c]	1105±73.2[a]	<0.001
IFN-γ, pg/mL	494.7±76.6[a]	297.0±39.8[b,c]	130.7±26.0[c]	148.9±16.1[c]	313.8±55.5[a,b]	<0.001
IL-1β, pg/mL	3754±348.3[a]	2187±136.8[a,b]	2125±306.1[b]	2960±292.5[a,b]	2918±139.1[a,b]	0.007
IL-6, pg/mL	258.3±51.5	156.9±62.4	161.6±38.5	214.4±27.3	163.9±28.6	0.411
IL-10, pg/mL	3014±428.1[a]	2455±194.2[a]	1121±103.0[b]	998.4±102.4[b]	1622±167.9[b]	<0.001
PHA (T cell mitogen)						
IL-2, pg/mL	234.0±25.2[a]	227.4±28.6[a]	116.3±12.5[b]	176.5±15.3[a,b]	220.3±20.3[a]	0.002
TNF-α, pg/mL	365.1±61.9	378.3±40.6	232.2±45.4	242.2±26.9	370.5±41.6	0.031
IFN-γ, pg/mL	77.6±17.7[a,b]	52.3±15.1[a,b]	37.7±10.2[b]	29.3±5.5[b]	102.2±19.7[a]	0.006
IL-6, pg/mL	160.2±21.3	121.7±25.7	93.9±40.8	94.4±21.4	190.5±24.2	0.059

IL-10, pg/mL	2422±448.3[a]	1693±235.5[a,b]	1052±183.0[b]	958.3±137.7[b]	1326±171.8[b]	0.001
PMA-I (T cell mitogen)						
IL-2, pg/mL	1343±282.4	1675±184.1	1056±197.4	1685±195.6	1686±207.2	0.138
TNF-α, pg/mL	3611±882.4[a,b]	3242±229.4[a,b]	1915±250.5[b]	3514±357.5[a,b]	4139±437.1[a]	0.013
IFN-γ, pg/mL	4207±491.4	3587±152.2	2366±744.0	3537±657.5	4383±261.3	0.178
IL-6, pg/mL	615.1±167.0[a]	279.6±66.8[a,b]	147.9±47.8[b]	114.2±30.2[b]	237.4±45.5[b]	0.009
IL-10, pg/mL	2185±437.8[a]	2035±84.8[a,b]	1095±181.8[b]	1319±142.6[a,b]	1652±152.3[a,b]	0.012

APC, antigen presenting cell; CHF, control high-fat diet; HFDairy, high-fat dairy diet; IFN-γ, interferon-gamma; IL, interleukin; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PMA-I, phorbol 12-myristate 13-acetate plus ionomycin; PWM, pokeweed mitogen; TNF-α, tumor necrosis factor-alpha. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

PBMC immune cell phenotypes

As presented in **Table 5**, the proportion of T cells (CD3+) were similar among all groups except for the LBW-LFDairy which had a slightly higher proportion compared to the NBW-CHF ($P < 0.05$). No change in the overall proportion of helper T cells and cytotoxic T cells were observed among groups. No other differences were observed among groups for any other T cell markers

including naïve T cells (CD3+CD45RA+) and helper and cytotoxic T cells expressing the IL-2 receptor (CD3+CD4+CD25+ and CD3+CD8+CD25+).

The proportion of APCs expressing toll-like receptor 4 (TLR-4, CD284+) was lower in both the LBW-CHF and LBW-HFDairy but not in the LBW-LFDairy compared with the NBW-Chow (all, $P < 0.05$). However, the proportion of monocytes expressing the TLR-4 (CD14+CD284+) and APCs expressing both major histocompatibility complex (MHC) class II and TLR-4 (SLAII+CD284+) were lower in all high-fat diet fed groups than in NBW-Chow (all, $P < 0.05$). No differences were observed among group for activated monocytes (CD14+CD11c+) and B cells (CD21+ SLAII+).

Table 5. PBMCs population of NBW and LBW swine fed different experimental diets

	NBW-Chow	NBW-CHF	LBW-CHF	LBW-HFDairy	LBW-LFDairy	*P*
% of gated cells						
Total CD3+ (T cells)	61.3±4.1[a,b]	59.6±1.7[b]	63.3±1.7[a,b]	65.5±2.3[a,b]	69.5±1.2[a]	0.032
CD3+CD4+ (Th)	62.4±2.4	61.0±2.9	64.8±2.0	59.3±2.9	59.2±2.5	0.519
CD3+CD8+ (cytotoxic T cell)	37.7±2.5	38.8±2.9	34.4±2.4	40.6±2.9	41.2±2.5	0.383
CD3+CD45RA+ (naïve T cell)	16.2±2.4	16.9±0.6	18.4±1.8	14.1±2.7	16.0±2.4	0.712
Total CD25+ (IL-2 receptor)	28.2±1.3	29.4±2.8	29.8±1.9	29.5±1.3	26.2±2.5	0.676
CD3+CD4+CD25+	30.5±1.1	28.2±4.2	36.1±4.5	40.0±4.9	30.0±3.7	0.257
CD3+CD8+CD25+	22.2±2.0	20.7±2.2	20.1±0.7	20.8±1.4	19.7±1.9	0.875
Total CD80+ (co-stimulator of T cell)	14.6±0.9	10.0±1.2	8.3±2.2	8.1±1.5	8.8±1.4	0.207
Total CD284+ (TLR-4)	3.9±0.4[a]	3.0±1.1[a,b]	1.2±0.3[b]	1.3±0.4[b]	2.5±0.6[a,b]	0.021
CD14+CD284+	25.2±3.6[a]	11.6±4.8[b]	9.8±2.5[b]	7.3±1.3[b]	9.8±1.6[b]	0.002
SLA+CD284+	11.0±0.8[a]	2.9±0.7[b]	2.7±0.8[b]	2.2±0.7[b]	3.0±0.8[b]	<0.001
CD14+CD11c+	8.7±2.4	8.2±3.4	7.7±2.3	5.9±2.1	3.6±1.1	0.502
CD21+ SLA+	15.2±3.5	9.2±0.9	18.5±2.7	17.4±4.0	16.8±3.1	0.503

CHF, control high-fat diet; HFDairy, high-fat dairy diet; IL, interleukin; LBW, low birthweight; LFDairy, low-fat dairy diet; NBW, normal birthweight; PBMC, peripheral blood mononuclear cell; Th, T helper cell; TLR-4, toll-like receptor 4. All values are expressed as means ± SEM. A p value was considered as statistically different when <0.05. Means sharing the same letter were not significantly different from each other.

Discussion

In the present study we assessed the intake of 3 servings/day of either low- or high-fat commercially available dairy products, that included milk, yogurt and cheese, on obesity-related cardiometabolic perturbations and immune function. We have confirmed that LBW piglets fed a high-fat diet exhibited early signs of insulin resistance and also demonstrated peripheral immune dysfunction. Feeding dairy products, regardless of fat content, was associated with improvements in fasting plasma glucose levels. Feeding low-fat dairy products improved peripheral immune function to a greater extent than high-fat dairy. These findings provide novel insights into the role of dairy products and dairy fat in modulating obesity-related cardiometabolic perturbations and immune dysfunction.

Dairy fat and its effect on choline moieties in PBMC's membrane and systemic inflammation

Intriguingly, our findings contradicted part of our initial hypothesis that high-fat dairy would potentially be more effective than low-fat dairy intake at improving immune function. This is likely in part due to the differences in choline content (and the forms of choline) between low- and high-fat dairy products. Unlike the structure of other dietary fats, milk fat droplets are normally enveloped by an amphiphilic membrane, namely milk fat globular membrane (MFGM). Polar lipids including PC, PE and SM are naturally found in dairy and specifically in the MFGM. During industrial processes such as making butter from raw milk, the MFGM together with polar lipids are disassociated from fat globules and preferentially enriched in the aqueous phase such as skimmed milk and buttermilk (Rombaut et al., 2006). Indeed, we have previously compared the choline content in 48 commercial dairy products in Canada and reported that total as well as both water- and lipid-soluble forms of choline were negatively

associated with total dairy fat content (Richard, Lewis, Zhao, et al., 2016). Our group has also recently demonstrated that buttermilk, as an important source of lipid soluble forms of choline (i.e., PC and SM), exhibited promising ability to support the immune system development and T cell function (Azarcoya-Barrera et al., 2020, 2021a). Therefore, the higher choline content in low-fat dairy products might explain, at least in part, the ameliorated immune function seen in the LBW-LFDairy group. We also assessed the two major phospholipid classes (PE and PC) in PBMCs membrane as an indirect marker of dietary PC intake. In rodents, we have previously shown that feeding a diet containing 100% PC as compared to a diet containing 100% free choline increased the PC content in splenocyte cell membranes which was associated with enhanced T cell function (Lewis et al., 2016). Here, we demonstrated that the PC:PE ratio in PBMCs increases in the context of high-fat diet feeding which can be normalized by dairy consumption with the most beneficial effect observed with low-fat dairy. Although there is limited evidence regarding the importance of the PC:PE ratio on immune function, a higher ratio has been linked to the progression of several metabolic diseases such as liver steatosis in *ob/ob* mice and insulin resistance in skeletal muscle (van der Veen et al., 2017). These data suggest that part of the greater beneficial effect that low-fat dairy has on immune function may be attributable to the higher amount of lipid soluble forms of choline normalizing the PC:PE ratio in the context of a high-fat diet.

The fact that high-fat dairy led to fewer changes in immune function than low-fat dairy in this model could also be due to the overall higher proportion of fat coming from the diet in this group. The proportion of fat coming from the 3 servings of high-fat dairy is roughly 64% whereas the proportion of fat in the low-fat dairy is about 26%. Therefore, by substituting some of the CHF diet that contains 46% fat by 3 servings of high-fat dairy, the overall proportion of fat

in the diet was increased to roughly 51%. It has been demonstrated that high-fat diet and obesity can lead to an increased gut permeability and endotoxin (LPS) translocation which in turn would trigger an inflammatory response (Duan et al., 2018; Massier et al., 2021; Mohammad & Thiemermann, 2021). Consistently, we demonstrated that feeding a high-fat diet in this swine model tended to increase circulating levels of inflammatory markers. On the other hand, in the context of a high-fat diet, providing a diet that contains a higher proportion of fat may have led to a higher endotoxin translocation and greater systemic inflammation as observed in the LBW-HFDairy group. Indeed, we have observed higher concentrations of circulating IL-1α, IL-1β and a trend for IL-1Ra in the LBW-HFDairy group. The higher systemic inflammation could be responsible in part for the lower immune function reported in this group as compared to LBW-LFDairy.

Dairy fat and its impact on glucose metabolism and insulin resistance

Although the proportions of total SFA, MUFA and PUFA in liver tissue were similar among all LBW groups, the proportion of C14:0 and C15:0 were higher in the LBW-HFDairy group. These SFAs have been used as biomarkers of dairy fat intake in humans (Abdullah et al., 2015a; Golley & Hendrie, 2014; Liang et al., 2018; Lund-Blix et al., 2016; Pranger et al., 2019). Particularly, C15:0 and C17:0, are odd-chain SFAs that cannot be endogenously synthesized that are specific to dairy and ruminant meat (Abdullah et al., 2015a). However, it has been previously reported that these two odd-chain SFAs are present in a variety of marine fish species (Özogul et al., 2009). Hence, it is not surprising that we have also detected C15:0 and C17:0 and higher proportion of EPA in liver tissue in the group that received the CHF diet since fish meal is added to the experimental diet. Both dairy groups consumed less of the experimental CHF diet to account for the energy intake coming from dairy consumption and therefore accumulated less

EPA. However, we confirmed that the biomarkers for dairy fat intake in humans are also relevant for this large animal swine model.

Meta-analyses of cohort studies have previously reported an inverse relationship between biomarkers of dairy fat intake, particularly the C15:0 and C17:0, and the incident of T2D in humans (Huang et al., 2019; Imamura et al., 2018a). *In vitro* studies have also reported that C15:0 play a direct role in glucose metabolism *via* promoting GLUT4 translocation to plasma membrane in myotubes (Fu et al., 2021). C15:0 has also recently been proposed to be an essential fatty acid due to a number of established cardiometabolic benefits including attenuation of glucose concentrations in mice fed with high-fat diet (Venn-Watson et al., 2020). Importantly, our findings in LBW swine model are in agreement with previous findings, suggesting that dairy fat may be responsible, at least to some extent, for the improvement in glucose metabolism in this model.

Dairy fat and its impact on dyslipidemia

Dairy fat is still a subject of controversy as it relates to its beneficial effect on cardiometabolic health due to its high content in SFA. Yet, emerging evidence from randomized controlled trials (RCT) mostly point to a neutral effect of dairy, irrespective of their fat content, on several cardiometabolic risk factors (J. P. Drouin-Chartier, Côté, et al., 2016). For instance, a recent RCT conducted by Schmidt et al. reported that consumption of 3.3 servings/day of either low- or high-fat dairy from the same food matrix, specifically milk, yogurt and cheese for 12 weeks, did not modulate serum concentrations of TC, LDL-C, HDL-C and TG in 72 subjects with metabolic syndrome (Schmidt et al., 2021). Consistently, in the current study, we also reported a neutral effect of dairy consumption, regardless of their fat content, on TC, LDL-C, HDL-C and TG

when compared to the LBW-CHF group. Altogether, our results suggest that feeding dairy products in the context of a high-fat diet has little effect on the lipid profile.

Dairy and their impact on peripheral blood T cell function

Previous findings in humans and rodents have demonstrated an impaired immune response to mitogen stimulation in the context of obesity and T2D (Azarcoya-Barrera et al., 2022; Lamas et al., 2002; Richard et al., 2017). Here, we found that consuming a high-fat diet in the context of a LBW swine model of insulin resistance also led to impaired immune function, particularly impaired T cell function. IL-2 is a cytokine that induces T cell proliferation and differentiation (Ross & Cantrell, 2018). Lower IL-2 production after PHA and PWM stimulation in the LBW-CHF suggests an impaired T cell proliferation and differentiation. Additionally, IFN-γ and TNF-α are two crucial cytokines produced by Th 1 cells known to increase IL-2 production. We know that IFN-γ upregulates antigen processing and presentation by APCs, modulates antiviral and antimicrobial activity and regulates leukocyte trafficking (Schroder et al., 2004). TNF-α induces fever and production of acute phase protein (Baud & Karin, 2001). Therefore, the lower IFN-γ and TNF-α production after PWM stimulation in LBW-CHF could explain, to some extent, the lower IL-2 production in this group. On the other hand, we have demonstrated that feeding low-fat dairy in the context of a high-fat diet exerted a greater ability to normalize IL-2, IFN-γ and TNF-α production after stimulation relative to the NBW-Chow group, suggesting that low-fat dairy may be more effective than high-fat dairy. Remarkably, we still observed that feeding high-fat dairy improved IL-2 after PHA, and TNF-α after PMA-I stimulation even though it was to a lesser extent than the low-fat dairy. Previous studies in humans have reported that individuals with obesity and T2D (when compared to individuals with obesity but metabolically healthy (MHO)) have higher proportions of naïve T cells (CD3+CD45RA+) and cytotoxic T cells despite

having similar proportions of total T cells (Richard et al., 2017). Moreover, individuals with obesity and T2D had a higher proportion of immune cells expressing activation markers such as CD80, and T cells expressing CD278, which play an important role in IL-2 production and T cell proliferation (Richard et al., 2017). In the current study, we did not observe any significant changes on the major T cell subsets or activation markers. A possible explanation is that although our model was characterized by early signs of insulin resistance, it did not lead to a state of frank T2D. Altogether, our data suggest that a high-fat diet impairs T cell function in LBW swine and that feeding low-fat dairy can counteract some of the obesity-related T cell dysfunction.

Dairy and their impact on peripheral blood APC function

IFN-γ and TNF-α can also be produced by APCs such as monocytes and dendritic cells. IL-1β is another crucial innate cytokine that belongs to the IL-1 family, which can be induced by nearly all microbial substances and causes inflammation (Dinarello, 2018). Therefore, lower levels of IFN-γ, TNF-α and IL-1β produced from LBW-CHF after PWM stimulation may also suggest an impaired innate immune response. Similarly, feeding low-fat dairy normalized the production of these cytokines in that they were no longer different from the NBW-Chow. Feeding high-fat dairy also normalized the production of IL-1β, but not the other cytokines. This could be explained, at least in part, by the differences in total cells expressing CD284+ (TLR-4) in PBMCs. TLR-4 is known to be one of the first lines of defense for our innate immune system by recognizing bacterial endotoxins and inducing subsequent immune responses (Molteni et al., 2016). Indeed, we reported that the expression of TLR-4 was lower in both the LBW-CHF and LBW-HFDairy groups while the LBW-LFDairy was similar to NBW-Chow. Decreased expression of TLR-4 has been previously reported in elderly patients who have a higher

prevalence of infection than younger patients (Schaenman et al., 2019). In contrast, B cell proportions were previously found to be similar in individuals with obesity and T2D compared to MHO (Richard et al., 2017). This is consistent with the current finding that the proportions of B cells and macrophages remained unchanged across all groups. Overall, our data suggest that a high-fat diet may also impair APCs function in LBW swine and that feeding low-fat dairy can improve APCs function by normalizing to some extent the expression of TLR-4.

As much as the acute inflammatory response to stimuli is important in mounting an adequate immune response, the resolution phase of inflammation is as important to prevent tissue damage. IL-10 is a key regulatory cytokine with anti-inflammatory properties and can be produced by a number of myeloid and lymphoid cells including Th2, regulatory T cells (Treg), dendritic cells and macrophages (Saraiva & O'Garra, 2010). The action of IL-10 on promoting the resolution phase of inflammation has also been previously reported (Takeda et al., 2015). In the present study, lower production of IL-10 in LBW-CHF after both T cell mitogen and APC mitogen stimulations is suggestive of an impaired anti-inflammatory cytokine production and overall diminished immune-suppressive ability. Indeed, serum concentrations of IL-10 were found to be significantly reduced in obese subjects and correlated with hyperinsulinemia and insulin resistance (Leon-Cabrera et al., 2015). IL-10 has also been found to be less effective at inhibiting inflammation in T2D patients, in a manner that failed to attenuate TNF-α production upon LPS stimulation (Barry et al., 2016). On the other hand, we demonstrated that feeding dairy, regardless of fat content, normalized to some extent the IL-10 production after PMA-I stimulation. Although this suggests that dairy may improve the resolution phase of inflammation, the enhanced IL-10 production cannot be attributable to a specific cell type (i.e., APCs, Th2 or Treg cells) nor inflammatory phase. Thus, future studies are warranted to validate which cell

type is responsible for the IL-10 production post-stimulation with more detailed laboratory assessment such as intra-cellular cytokine staining technique.

Strength and limitations

To the best of our knowledge, this is the first study using a swine model of obesity and insulin resistance to investigate the effect of 3 servings/day of low- *vs* high-fat dairy products using similar food matrices on cardiometabolic risk factors and immune function. This is important since most studies in the field trying to understand the effect of dairy fat *per se* have compared milk or yogurt to cheese and butter which do not take into consideration the importance of the food matrix. The higher-than-expected inter-individual variations in some outcomes (i.e., insulin) suggest that future studies should increase the sample size and perhaps the duration of the intervention to induce a stronger insulin-resistant phenotype.

Conclusion

In conclusion, findings from the present study provide new mechanistic evidence that support the role of dairy products, specifically milk, yogurt and cheese, in counteracting some of the cardiometabolic and immune dysfunction associated with obesity. Consumption of 3 servings per day of high-fat dairy products improved (lowered) fasting glucose more than low-fat dairy, whereas low-fat dairy improved immune function particularly T cell function to a greater extent than high-fat dairy. Irrespective of fat content, consumption of 3 servings of dairy products had a neutral effect on the lipid profile in this swine model of insulin resistance.

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Appendix C. Muscle fiber, connective tissue and meat quality characteristics of pork from low-birth-weight pigs as affected by diet-induced increased fat absorption and preferential muscle marbling

Appendix C is a research paper that investigates how birth weight difference in piglets affects carcass properties as well as pig meat quality at slaughter age. The manuscript is in preparation and will be submitted to *Canadian Journal of Animal Science.*

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Introduction

Genetic selection strategies to increase prolificacy in polytocous pigs resulted in increased litter size which ultimately reduced the mean birth weight of piglets within-litter (Milligan et al. 2002; Quiniou et al. 2002) and led to intra-uterine growth retardation of the embryos due to limited placental capacity (Stange et al. 2020; Baur et al. 1998). It has been shown that low birth weight (LBW) piglets (0.95 to 1.3 kg) exhibit reduced post-natal growth rate and finally lower lean carcass percentage than normal birth weight (NBW) piglets (1.4 to 1.6 kg) at slaughter (Bee, 2004; Gondret et al. 2006; Morise et al. 2008). Total number of muscle fibers in a muscle is an important determinant for the total mass of a muscle (Luff and Goldspink 1970; Miller et al. 1975) because fast growing pig strains tend to have a higher number of muscle fibers in their

muscles than their slow growing counterparts (Ezekwe and Martin 1975; Miller et al. 1975). There is some evidence that LBW (runt) piglets tend to grow more slowly and less efficiently than their NBW (larger) littermates (Powell and Aberle 1980). As a result, piglets with LBW require a longer growth period than do the NBW piglets or their heavier littermates to reach the same slaughter weight (Wolter et al. 2002) and their growth is characterized by a lower feed efficiency that ultimately results in reduced rate of weight gain as well as a lower lean percentage carcass and meat quality than NBW pigs at slaughter (Gondret et al. 2005a; Rehfeldt and Kuhn 2006). Moreover, birth weight in pigs is related to post-natal muscle development as well as fat accretion, and ultimately meat quality (Bee, 2004; Poore and Fowden 2004; Gondret et al. 2005b) though contradiction exist (Bérard et al., 2008). Chemical analysis revealed that at slaughter, muscles of LBW piglets contain lower intramuscular fat and protein but higher water than their NBW littermates (Rehfeldt and Kuhn, 2006) and this difference was more pronounce in locomotive (*semitendinosus*) muscle than in postural (*longissimus*) muscle (Gondret et al., 2005b) although contradiction exists for intramuscular fat content (Rehfeldt et al., 2008). In practice, LBW piglets are grown by fostering or feed them individually from birth to slaughter which might cause the carcass to be fatter and have an increased intramuscular fat content compared with their NBW counterparts (Powell and Aberle 1980). At weaning, LBW piglets weighed 12 % less than NBW piglets and required 12 more days to reach slaughter weight (Gondret et al. 2005b).

The total number of muscle fibers in a muscle is lower in LBW piglets than NBW piglets or their heavier littermates, a characteristic that is fixed at birth (Wigmore and Stickland 1983). Although they have fewer muscle fibers, the muscle fibers they do have are larger in mean diameter / cross-sectional area at slaughter weight (Kuhn et al. 2002; Gondret et al. 2005b)

which might contribute to increased meat toughness in *longissimus* muscle as tenderness score and muscle fiber diameter is negatively correlated (Gondret et al. 2006). However, Maltin et al. (1997) did not find any relationship between muscle fiber cross-sectional area and meat tenderness in pork. The variation in total muscle fiber number in relation to piglet birth weight is not always consistent (Dwyer et al. 1993). It was shown that muscles with a lower total muscle fiber number that have large mean diameter/ cross-sectional areas are prone to rapid post-mortem pH decline and high drip losses that ultimately alter meat tenderness (Lengerken et al. 1997) and lead to pale, soft exudative pork (Rosenvold and Andersen 2001). Muscle characteristics such as muscle fiber type composition, intramuscular fat content, total collagen content and collagen heat-solubility differ with birth weight and influence meat quality (Lebret et al. 1999).

During fetal and early post-natal stages, the development of tissues in the animal body prioritize according to the nutrient supply. It has been demonstrated that ovine fetuses when exposed to undernutrition in utero possess more intramuscular fat in their muscle (Bispham et al. 2003, 2005; Symonds et al. 2003). It was suggested that when muscle is unable to form, intramuscular fat and connective tissue components increase in muscle as a default pathway (Kablar et al. 2003). It is well accepted that LBW piglet (< 0.93 kg) might reduce post-natal muscle development but increase adipose tissue development during their lifetime (Gondret et al. 2006). From the above discussion, we hypothesized that LBW piglets require longer time to reach slaughter weight due to their lower growth rate and produce carcasses with lower yields of lean with inferior quality. The objective of this study was to investigate the influence of birth weight of piglets on overall growth performance, carcass components and traits and muscle fiber characteristics, intramuscular fat content, collagen characteristics in the intramuscular connective tissue of *longissimus* muscle and the consequences on meat quality when piglets were fed dairy

and non-dairy sources of fat.

Materials and Methods

Animals and diets

Newborn male piglets (Landrace Large White × Duroc) were weighed within 24 h of full-term birth and were identified as normal birth weight (NBW, 1.89 ± 0.02 kg, n = 11) and low birth weight (LBW, 1.05 ± 0.04 kg, n =13). The piglets were then assigned randomly within birth weight classification to receive the NBW-control diet (NBW-C; 1.62 ± 0.07 kg SEM) or NBW-high fat diet (NBW-HF; 1.73 ± 0.09 kg SEM) and LBW-high fat diet (LBW-HF; 1.29 ± 0.08 kg SEM) or LBW-high fat from dairy source diet (LBW-HFHD; 1.18 ± 0.06 kg SEM). From birth to weaning (3 weeks of age) piglets received maternal milk and a standard grower diet up to 5 weeks of age. At 5 weeks of age, all piglets were fed a 1-week transition diet and then NBW piglets were randomly assigned to control (NBW-C, n = 5) or high fat (HF) diet group (NBW-HF, n = 6) and LBW piglets were randomly assigned to either high fat (HF) diet (LBW-HF, n = 8) or isocaloric diet (3 servings/2000 kcal/day) of full fat dairy products (LBW-HFHD, n = 5) and fed these diets for a 6-week dietary experimental feeding period. Details regarding control chow and high fat diet have been described previously (Fontaine et al., 2019). Briefly, energies from fat, protein and carbohydrates were 14 %, 17 % and 69 % respectively in control diet, while 46 % (mainly lard), 21 % and 33 % (mainly fructose) respectively in high fat diet. 1 % cholesterol was added as well in the high fat diet. Each serving of dairy products contained whole milk powder (3.25 % fat, 33g; Bulk Barn, Canada), plain yogurt (10 % fat, 175 ml; Liberté, Canada) and mozzarella cheese (28 % fat, 50 g; No name®, Canada). Piglets for both NBW and LBW treatments were obtained from different dams and live weight was recorded at

birth and then weekly until 12 weeks of age (slaughter age).

Slaughter and carcass characteristics

At 12 weeks of age, pigs were euthanized by captive bolt and slaughtered by exsanguination and the carcasses then eviscerated. A blow torch was used to remove the hair from the carcasses and then the carcasses were transported by truck to the food laboratory to Agri-Food Discovery Place (AFDP University of Alberta, Edmonton, Canada) within 10 minutes. Upon arrival at the laboratory, the carcasses were washed with cold tap water and the hot carcass weight (HCW) was recorded and then the carcass was taken into a room chiller (4 °C), hung, and allowed to cool for 24 hours. After 24 hours, the cold carcass weight and carcass length (from the first cervical vertebrae bone atlas to base of the tail) was recorded. The carcass was then cut into halves and both sides were dissected into primal cuts (pork shoulder, pork belly, pork loin and pork legs) following the Canadian Food Inspection Agency Meat Cuts Manual (https://inspection.canada.ca/food-label-requirements/labelling/industry/meat-and-poultry-products/meat-cuts/eng/1300126276015/1300126349342). The weights of each cut were recorded, and their yields calculated as percentages of the hot carcass weight. The thoracic region of pork loin was removed from the right side of the carcass, weighed, and retained for measurement of meat quality at 24 hours post-mortem. How the pork loin thoracic region was fabricated for meat quality analysis is illustrated in **Figure 1**. One chop (**Figure 1**, steak A at 2 days post-mortem) was used to measure subcutaneous back fat depth and loin muscle depth following Teixeira et al. (2021) and was described elsewhere in details (Wang et al., under review), which were used to calculate the Canadian Lean Yield percentage as described by Pomar and Marcoux (2003).

Canadian Lean Yield (CLY, (%) = 68.1863 − (0.7833 × fat depth in mm) + (0.0689 × muscle depth in mm) + (0.0080 × fat depth in mm^2) − (0.0002 × muscle depth in mm^2) + (0.0006 × fat depth in mm × muscle depth in mm).

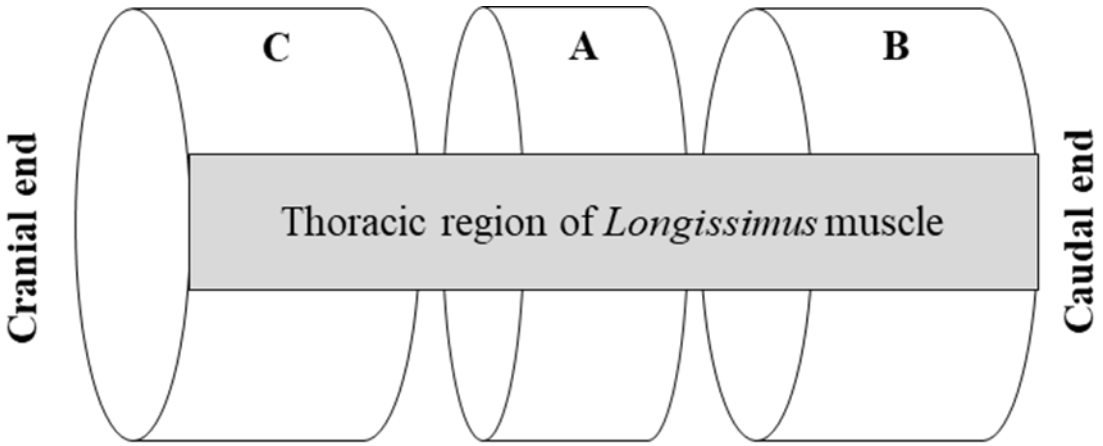

Fig. 1. Breakdown of loin eye muscle from right side of the pork carcasses for meat quality characteristics determination at 24 h post-mortem (thoracic region: A, B and C). One chop (about 2.54 cm) from thoracic region (A) was used for objective meat color, subcutaneous (backfat) fat depth and loin muscle depth to calculate *longissimus* muscle area and the drip loss measurement. Three ribs section from thoracic region (B) were used for cooking, cooking loss measurement and Warner-Bratzler shear force determination after cooking. Four ribs section from thoracic region (C) were used for muscle fiber typing. After samples taken for muscle fiber typing chop (C) was trim for epimysium, cut into small cubes, freeze and then lyophilized, ground and used for determination of proximate components and collagen solubility.

Pork meat quality characteristics

Pork meat quality characteristics were determined using loin eye muscle as described in **Figure 1**.

Meat color

Meat color was determined on a freshly cut cross sectional surface of the chops from *longissimus thoracis* muscle after the surface had been exposed to air (bloom) for 20 minutes at

room temperature. Surface color was measured using a CR-400 chroma meter (Konica Minolta Sensing, Inc., Japan) in CIE standard color system where lightness (L*; ranging from 0 for black to 100 for ideal white), redness (a*; color coordinate where positive values indicates redness and negative values indicates greenness), yellowness (b*; color coordinates where positive values indicates yellowness and negative values indicates blueness), and color intensity or saturation (C*; chroma and h*; hue) index (Commission Internationale de I'Eclairage 1986) were recorded. The diameter of the aperture area was 8 mm with a 2° standard observer angle, and the D65 illuminant setting was used. The chroma meter was calibrated before the measurement using a white calibration ceramic tile provided by the manufacturer and the readings of the standard white tile were L* = 93.21, a* = 0.3126, b* = 3192. Three readings were performed on each chop, and the mean of the three readings used for statistical analysis. Readings were performed on the lean portion of each chop, avoiding areas of intramuscular fat and connective tissue.

Intramuscular ultimate pH 24h

An Accumet AP71 pH meter (Fisher Scientific, Mississauga, Ontario) was used to measure the ultimate pH at 24 h postmortem with a pH electrode that was temperature compensated (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON) and calibrated with commercial standards of pH 4.0 and 7.0. The glass electrode was inserted into cuts made in the muscles using a knife as along with the temperature probe. The average of three readings was calculated and used for statistical analysis.

Drip loss

Drip loss was determined following the standard bag method according to the method of Honikel (1994). For this, approximately 50-60 g of *longissimus thoracis* muscle trimmed of epimysium was suspended in an inflated plastic bag with a metal hook for 24 hours at 4 °C. Drip

loss was considered and calculated as the weight loss during the 24 h suspension period as a percentage of the initial weight of the muscle.

Cooking of meat and cooking loss determination

After color measurement, the chops were weighed, penetrated with a thermocouple (Tiny-Tag View 2S, Gemini Data Loggers, Chichester, West Sussex, UK) at the geometrical center of the steak to monitor the cooking temperature and then cooked on an electric clam-shell style grill (General Electric 4 in 1 Grill/Griddle, China) at 165 ℃ (325 °F). Chops were cooked until the temperature at the geometrical center of each chop reached 71 °C. At 71 °C, the cooked chop was immediately removed from the grill, placed in a plastic bag, and immersed in ice water to stop the cooking process. Then, the evaporated and condensed water in the plastic bag was poured off and chops were stored overnight in the plastic bag at 4 °C. The following day, the chops were patted dry with paper towels and weighed to determine the cooking loss by the equation:

$$\text{Cooking loss (\%)} = \frac{\text{(Meat chop weight before cooking – Meat chop weight after cooking and patted drying)}}{\text{Meat chop weight before cooking}} \times 100$$

Warner-Bratzler shear force determination

For measurement of Warner-Bratzler shear force (WBSF), the cooked chops were kept at room temperature (22-25 °C) about 30 minutes for temperature equilibrium. Then, six cores of 1.27 cm diameter were made with a metal cork borer parallel to the muscle fibers direction from each cooked steak avoiding thick visible fat or connective tissue. Peak force was measured using a V-shaped shear blade attached to a material testing machine (Lloyd Instrument LRX plus, AMETEK®, Digital Measurement Metrology Inc. Brampton, ON) at a pre-load force of 2 N and

a cross-head speed of 200 mm/min. Mean of the six recorded peak shear force values expressed as Newtons was obtained and used for statistical analysis.

Proximate composition of meat

Moisture

About 100 g of a raw meat steak was trimmed of epimysium and cut into small cubes that were evenly distributed in a small aluminum tray, covered with perforated aluminum foil, and then frozen at -20 °C until lyophilized for 5-7 days. After lyophilization the weight loss was reported as the moisture content (as a percentage of total raw meat weight) and was calculated by the equation:

$$\text{Moisture (\%)} = \frac{\text{(Raw meat weight – Lyophilized meat weight)}}{\text{Raw meat weight}} \times 100$$

Crude fat

The crude fat content was measured with the Soxtec™ 2050 fat extraction (Foss® Analytical, Hilleroed, Denmark) apparatus by following the method of Roy et al. (2018) according to the Association of Analytical Communities Official Method of Analysis, 991.36 (Thiex et al. 2003). About 2.0 grams of lyophilized ground meat were weighed into a cellulose thimble (33 × 80 mm, Foss™ Thimbles for Soxtec™ 2055 Manual Fat Extraction System, Fisher Scientific, Cat No. TC15220045) and packed with defatted cotton (Foss™ Accessories for Soxtec™ Extraction Systems: Fisher Scientific, Catalog No.TC15290009). The extracted fat was calculated as percentage on raw meat basis using the following equation:

$$\text{Crude fat (\%)} = [\text{Fat in lyophilized meat (\%)} \times \text{Dry matter (\%) in lyophilized meat}] \div 100$$

Crude protein

Crude protein was determined as described in the AOAC (1990) method using a LECO FP-2000 Nitrogen Determinator (Leco Corp., St. Joseph, MI). About 100 mg of lyophilized ground meat was weighed into a Foil Boat liner (Cat. No. 502-343, LECO) and the weight recorded. Standards, blank and calibration procedures were performed using LECO TruMac N with TruMac operating software as described in the manufacturer operator instruction manual. Ethylenediaminetetraacetic acid (EDTA, 9.65 % nitrogen, Leco Corporation) was used as a standard for calibration procedures and was analyzed after every 20 samples. Helium was used as a carrier gas, and nitrogen measured with a thermal conductivity detector and then converted to the concentration of protein using the conversion factor 6.25, which assumes that meat protein contains 16 % nitrogen.

Ash

Approximately 2.0 grams of lyophilized ground meat were weighed into pre-dried and pre-weighed Pyrex glass bottles. The glass bottles with lyophilized ground meat were then placed into an oven for 24 h at 110 °C and then into a furnace at 490 °C for 24 h to incinerate the meat into ash. Then, the glass bottles with ash were cooled at room temperature in a desiccator and weighed. The content from the lyophilized ground meat remain after incineration into the furnace was ash and calculated as percentage on raw meat basis according to the following equation:

$$\text{Crude ash (\%)} = [\text{Ash in lyophilized meat (\%)} \times \text{Dry matter (\%) in lyophilized meat}] \div 100$$

Total, soluble, and insoluble collagen determination

Soluble and insoluble collagen fraction separation

Soluble collagen was extracted from lyophilized ground meat following the Hill (1966) method. About 2.00 grams of lyophilized meat were weighed, the weight recorded, and then the meat heated with a total of 18 mL ¼ Ringer's solution at 77 °C for 1 h in a 25 mL Teflon capped glass tube. Following heating, the tubes were cooled to room temperature, and then the supernatant (soluble collagen) and residue (insoluble collagen) collected using centrifugation at 3500 *g* for 10 minutes. Extractions were performed in duplicate and means are used for statistical analysis.

Hydrolysis of soluble and insoluble collagen fraction and preparation for hydroxyproline assay

Two 1 mL aliquots of supernatant (for soluble collagen) and about 0.30 g of residue (for insoluble collagen) were hydrolyzed in 6 mL of 6 M HCl for 20 h in a 20 mL glass Teflon-capped test tube. Following hydrolysis, tubes were cooled to stop hydrolysis and then filtered (Whatman No. 4 filter paper, Fisher Scientific, Edmonton, AB). Filtered hydrolysates were evaporated to dryness, reconstituted with deionized water, and neutralized with NaOH. After neutralization, the reconstituted hydrolysates were evaporated to dryness, reconstituted with 5 mL of deionized water, and used for hydroxyproline assay to determine the soluble and insoluble collagen.

Hydroxyproline assay for collagen estimation

Hydroxyproline content was determined following the method of Bergman and Loxley (1963) for the quantification of soluble and insoluble collagen.

For hydroxyproline determination, 1.0 mL of soluble or insoluble hydrolysate was used. The blank was prepared in the same manner as samples by using deionized water (1 mL). Absorbance

was measured against the blank at 558 nm. Hydroxyproline standards (trans-4-Hydroxy-L-proline, Sigma-Aldrich) with concentrations of 2.5, 5.0, 10, 20 and 40 µg hydroxyproline/ mL solutions were prepared. The hydroxyproline concentration in the sample was calculated using a standard curve determined by regressing the concentration of each standard against its absorbance and then multiplying the concentration by the dilution factor for each sample. Hydroxyproline content was calculated and based on the calculation of Stanton and Light (1987), with hydroxyproline content converted into collagen concentration (mg/ g raw meat) using a conversion factor of 7.14. Total collagen was calculated by adding soluble and insoluble collagen together and collagen solubility was calculated with the formula:

$$\text{Collagen solubility (\%)} = \frac{\text{Soluble collagen (mg/ g raw meat)}}{\text{Total collagen (mg/ g raw meat)}} \times 100$$

For each sample, duplicates were performed and their mean used for statistical analysis.

Muscle fiber type determination

Muscle samples for muscle fiber typing with histological and immuno-histological characterization of the *longissimus* muscles was conducted by collecting 1 cm^3 muscle samples from each muscle at fabrication 24 h post-mortem. Muscle cubes were immersed in acetone cooled with dry ice as described by Roy et al. (2018). The muscle cubes were then stored at -80 °C until further processing. The muscles cubes were sectioned transversely (10 µm thick) with the muscle fiber direction in a cryostat (Leica CM1850 cryostat, Leica Biosystems Nussloch, Germany) at -25 °C and serial sections were mounted on dry slide glass and stored at – 80 °C until staining. At staining, mounted muscle sections were removed from storage and air-dried at room temperature

for 30 minutes. Muscle fiber typing was performed using the myofibrillar adenosine triphosphatase (mATPase) staining method (Brooke and Kaiser 1970) and the nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase (NADH-TR) staining method (Roy et al. 2018). Confirmation of type I muscle fiber type was performed by immuno-fluorescence histochemistry using monoclonal antibodies (S58, skeletal muscle myosin antibody from Santa Cruz Biotechnology, Inc.) specific for the type I myosin isoform (Roy et al. 2018). Myofibrillar adenosine triphosphatase (mATPase) staining was performed on muscle sections after pre-incubation in acid (pH 4.3) and in alkali (pH 10.5). The classification of muscle fiber typing was performed as presented in **Figure 2** by following the different staining procedures in serial sections as mentioned above. Using the open-source software ImageJ (http://rsbweb.nih.gov/ij/), cross-sectional areas of muscle fibers were measured from three randomly captured images at 200x magnification, which included at least 300 fibers from each sample and were converted to diameter. To determine the muscle fiber diameter class interval relative to frequency percentages, different class intervals of the diameter (10-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 and 81-90 μm) of total muscle fibers counted for diameter from each treatment group were considered.

Statistical analysis

Data were analyzed by R (version 3.6.1) using the package lessR using one-way analyses of variance (ANOVA) where treatments (birth weight) were considered as the sole source of variation. The Tukey's Honestly Significant Difference (HSD) test was used to detect significant differences between means when significant main effects were detected. All comparisons with $P < 0.05$ were considered statistically significant and $0.05 < P < 0.10$ were considered to approach significance. Muscle fiber diameter class interval frequency percentages were analyzed by one-way analysis of

variance (ANOVA) within each diameter interval class using piglets birth weight groups as the sole source of variation. Where the model was significant ($P < 0.05$), Tukey's HSD test was used to determine mean differences between the treatments.

Results

Live weight of the piglets with age

The weekly mean live weights of the piglets by treatment are presented in **Table 1**. As planned, the birth weight of the piglets was significantly higher ($P = 0.0003$) in both NBW treatments (NBW-C and NBW-HF) compared with both LBW treatments (LBW-HF and LBW-HFHD). The NBF-HF pigs had heavier mean body weights than the LBF-HF and LBF-HFHD pigs up to 4 weeks of age, but by 5 weeks of age, mean body weights across the treatments were not different ($P > 0.05$). From 6 to 12 weeks, LBW pigs fed high fat diets (LBW-HF) had mean body weights similar to that of NBW pigs at 9, 10 and 12 weeks of age (**Table 1**). LBW-HFHD pigs had mean live weights similar to that of NBW-C and LBW-HF pigs at weeks 8, 9, 10 and 12.

Table 1. Live weight of piglets at birth and weekly with increasing age in different treatment groups

Age (Weeks)	Treatment groups				P-value
	NBW-C	NBW-HF	LBW-HF	LBW-HFHD	
n	**5**	**6**	**8**	**5**	
At birth	1.62 ± 0.07a	1.73 ± 0.09a	1.29 ± 0.08b	1.18 ± 0.06b	0.0003
1	2.94 ± 0.30 ab	3.57 ± 0.39a	2.50± 0.33ab	2.10 ± 0.16b	0.0351
2	4.5 ± 0.34ab	5.52 ± 0.59a	3.75 ± 0.49b	3.74 ± 0.21ab	0.0470
3	6.58 ± 0.29ab	7.52 ± 0.43a	5.65 ± 0.57b	5.36 ± 0.40b	0.0234
4	9.08 ± 0.74ab	9.95 ± 0.42a	7.31 ± 0.81b	6.24 ± 0.13b	0.0045
5	11.22 ± 1.23xy	13.05 ± 1.17x	9.51 ± 1.03y	8.90 ± 0.32y	0.0502
6	14.44 ± 1.57	15.33 ± 1.70	12.51 ± 1.46	12.40 ± 0.52	0.4237
7	19.60 ± 1.64	21.52 ± 1.87	16.91 ± 1.90	15.70 ± 0.70	0.1252
8	25.92 ± 2.03xy	28.87 ± 2.10x	23.95 ± 2.02y	21.12 ± 1.23y	0.0884
9	32.36 ± 2.08xy	35.38 ± 2.21x	29.57 ± 2.40xy	26.82 ± 1.10y	0.0788
10	37.66 ± 2.27xy	43.48 ± 2.54x	37.08 ± 2.73xy	32.54 ± 1.21y	0.0537
11	42.78 ± 2.93	46.85 ± 2.29	41.59 ± 2.71	37.34 ± 1.25	0.1223
12	48.44 ± 2.23xy	53.62 ± 1.97x	48.10 ± 2.05xy	45.64 ± 1.22y	0.0960

Data represents Mean ± standard error (SE)
Different letters (a, b) in the same row are significantly different in different animal groups at the 0.05 level of probability ($P < 0.05$).
Different letters (x, y) in the same row indicate differences approached significance ($0.10 > P > 0.05$) in different animal groups
NBW = Normal birth weight; LBW = Low birth weight; C = Control (chow diet); HF = High fat diet; HFHD = High fat dairy diet
The dark shaded area of the table shows that the transitional diet was provided
The light shaded area of the table shows that the experimental diets were provided

Carcass characteristics

The carcass composition of the pigs at slaughter from the different treatments is presented in **Table 2**. Hot carcass weights were not different between the treatments, with NBW-HF pigs tending to be heavier than LBW-HFHD pigs (P = 0.0615). Cold carcass weights were not different due to treatment, nor was carcass length, *longissimus thoracis* muscle depth and area, dressing percentage, pork shoulder weight percentage, pork loin weight percentage or pork belly weight percentage (**Table 2**). Mean subcutaneous fat depth was lower in the LBF-HFHD pigs than in the NBF-HF and LBW-HF pigs but did not differ from the NBF-C pigs which indicated that inclusion of dairy fat as a fat source in the diet of pigs significantly decreased subcutaneous fat depth (P = 0.0229). The pork leg proportion was significantly higher in LBF-HFHD than in NBF-C pigs but did not differ from pigs fed the other HF diets. The mean Canadian lean yield (CLY) percentage was significantly higher in LBW-HFHD pigs compared with pigs receiving other HF diets regardless of birth weight but not different from that of NBW-C pigs.

Table 2. Effect of birth weight of piglets on carcass characteristics of pork at 24 h post-mortem

Parameters	NBW-C	NBW-HF	LBW-HF	LBW-HFHD	P-value
n	**5**	**6**	**8**	**5**	
Hot carcass weight (kg)	37.08 ± 2.40xy	40.77 ± 2.27x	36.26 ± 1.49xy	33.44 ± 2.40y	0.0615
Cold carcass weight (kg)	34.84 ± 2.66	39.07 ± 2.52	35.23 ± 1.65	33.26 ± 2.66	0.2248
Carcass length (cm)**	77.50 ± 2.18	79.68 ± 2.06	75.78 ± 1.35	76.00 ± 2.18	0.2749
Subcutaneous fat depth (cm)	1.21 ± 0.17bc	1.45 ± 0.16ab	1.40 ± 0.10ab	0.90 ± 0.17c	0.0229
Longissimus muscle depth (cm)	3.76 ± 0.42	4.62 ± 0.39	4.56 ± 0.26	4.56 ± 0.42	0.1925
Longissimus muscle area (cm^2)	11.56 ± 2.73	17.18 ± 2.59	15.36 ± 1.70	19.03 ± 2.73	0.1140
Canadian lean yield (CLY, %)	62.51 ± 0.82ab	61.69 ± 0.78b	62.00 ± 0.52b	64.79 ± 0.82a	0.0085
Dressing (%) on HCW basis	73.57 ± 2.81	76.17 ± 2.66	72.37 ± 1.74	73.28 ± 2.81	0.5592
Pork shoulder weight (%) on HCW basis	29.99 ± 2.11	30.12 ± 2.00	26.88 ± 1.31	27.50 ± 2.11	0.3083
Pork leg weight (%) on HCW basis	21.70 ± 3.10b	22.99 ± 2.94ab	29.22 ± 1.93ab	31.97 ± 3.10a	0.0148
Pork loin weight (%) on HCW basis	25.33 ± 1.64	25.08 ± 1.56	22.27 ± 1.02	22.79 ± 1.64	0.1773
Pork belly weight (%) on HCW basis	16.77 ± 0.81	17.52 ± 0.77	18.01 ± 0.50	17.20 ± 0.81	0.4824

Data represents Mean ± standard error (SE)
Different letters (a, b, c) in the same row are significantly different in different animal groups at the 0.05 level of probability ($P < 0.05$).

Different letters (x, y) in the same row indicate differences approached significance (0.10 > P > 0.05) in different animal groups
** Carcass length was measured from the first cervical vertebrae bone atlas to base of the tail.
NBW = Normal birth weight; LBW = Low birth weight; C = Control (chow diet); HF = High fat diet; HFHD = High fat dairy diet; HCW = Hot carcass weight

Physical properties of pork meat from Longissimus thoracis muscle at 24 h post-mortem

Meat quality characteristics were determined in *longissimus thoracis* muscle and are presented in **Table 3**. There were no significant differences in the color parameters lightness (L*), redness (a*), chroma, hue, ultimate pH 24 h, Warner-Bratzler shear force, and cooking loss of the meat due to treatment. Pork from LBW-HFHD pigs tended to have a higher yellowness (b*) value than that of NBW-C pork but did not differ from pork from the NBW-HF and LBW-HF pigs (P = 0.0587). Drip loss also tended to be higher for pork from NBF-HF pigs than in that from NBF-C pigs but did not differ with LBF-HF and LBF-HDHF groups (P = 0.0985).

Table 3. Physical properties of pork meat from *Longissimus thoracis* muscle at 24 h post-mortem from different birth weight groups

Parameters	**NBW-C**	**NBW-HF**	**LBW-HF**	**LBW-HFHD**	**P-value**
n	**5**	**6**	**8**	**5**	
Lightness (L*) values	50.17 ± 1.27	53.41 ± 1.20	52.12 ± 0.79	52.83 ± 1.27	0.1311
Redness (a*) values	8.14 ± 0.48	8.32 ± 0.46	8.25 ± 0.30	8.08 ± 0.48	0.9649
Yellowness (b*) values	0.95 ± 0.49y	1.54 ± 0.46xy	1.53 ± 0.30xy	2.52 ± 0.49x	0.0587
Chroma values	8.21 ± 0.51	8.56 ± 0.49	8.44 ± 0.32	8.42 ± 0.51	0.9331
Hue values	6.59 ± 3.08	10.34 ± 2.92	9.98 ± 1.91	15.03 ± 3.08	0.1359
Ultimate pH 24 h	5.53 ± 0.06	5.52 ± 0.05	5.47 ± 0.03	5.52 ± 0.06	0.6784
Drip loss (%)	1.87 ± 0.95y	4.40 ± 0.90x	3.43 ± 0.59xy	2.54 ± 0.95x	0.0985
Cooking loss (%)	19.91 ± 1.86	18.02 ± 1.76	18.60 ± 1.15	16.82 ± 1.86	0.5138
Warner-Bratzler shear force (N)	31.86 ± 3.43	25.94 ± 3.25	30.94 ± 2.13	26.55 ± 3.43	0.2595

Data represents Mean ± standard error (SE)
Different letters (x, y) indicate differences approached significance (0.10 > P > 0.05) in different animal groups
NBW = Normal birth weight; LBW = Low birth weight; C = Control (chow diet); HF = High fat diet; HFHD = High fat dairy diet

Proximate composition and collagen content of pork

Both the proximate composition and collagen content of pork were determined in *longissimus thoracis* muscle and results are presented in **Table 4**. There were no significant differences between birth weight treatments for muscle crude protein, intramuscular fat, ash, total collagen, soluble collagen, insoluble collagen content and collagen solubility percentage. Moisture content was significantly higher in the pork from NBF-C pigs than NBF-HF and LBF-HF pigs but did not differ from that of LBW-HDHF pigs. The mean percentage of soluble collagen in pork from NBW-C pigs tended to be higher than that of NBW-HF pigs, but this difference only approached significance (P = 0.0673).

Table 4. Proximate composition and collagen content of pork from *Longissimus thoracis* muscle at 24 h post-mortem from different birth weight groups

Parameters	NBW-C	NBW-HF	LBW-HF	LBW-HFHD	P-value
n	**5**	**6**	**8**	**5**	
Crude protein (%)	20.97 ± 0.26	21.12 ± 0.25	21.08 ± 0.16	20.86 ± 0.26	0.7955
Moisture (%)	75.82 ± 0.33a	74.67 ± 0.32b	74.89 ± 0.21b	75.11 ± 0.33ab	0.0229
Intramuscular fat (%)	1.59 ± 0.37	2.40 ± 0.35	2.38 ± 0.23	2.51 ± 0.37	0.1213
Ash (%)	1.17 ± 0.03	1.12 ± 0.03	1.16 ± 0.02	1.17 ± 0.03	0.2375
Total collagen (mg/ g raw meat)	3.57 ± 0.50	3.20 ± 0.47	2.72 ± 0.31	2.40 ± 0.50	0.1764
Insoluble collagen (mg / g raw meat)	2.36 ± 0.41	2.27 ± 0.39	1.86 ± 0.25	1.71 ±0.41	0.3873
Soluble collagen (mg / g raw meat)	1.21 ± 0.17x	0.93 ± 0.16xy	0.86 ± 0.10xy	0.69 ± 0.17y	0.0673
Collagen solubility (%)	35.24 ± 1.45	28.87 ± 4.22	31.62 ± 2.76	28.63 ± 4.45	0.5035

Data represents Mean ± standard error (SE)
Different letters (a, b) in the same row are significantly different in different animal groups at the 0.05 level of probability ($P < 0.05$).
Different letters (x, y) in the same row are approached to significantly different in different animal groups at the 0.05 level of probability ($P > 0.05$)
NBW = Normal birth weight; LBW = Low birth weight; C = Control (chow diet); HF = High fat diet; HFHD = High fat dairy diet

Muscle fiber characteristics

Muscle histological cross-sections depicting the three muscle fiber types (I, IIA, and IIB) from pigs receiving different dietary treatments are illustrated in **Figure 2** and their properties are presented in **Table 5**. The total muscle fiber number in the whole cross-sectional area of the *longissimus thoracis* muscle was not significantly different due to treatment, nor was the percentage of different muscle fiber types. Light birth weight piglets (LBW-HFHD) had a significantly greater mean muscle fiber diameter compared to NBW piglets receiving either

control or HF diets. The LBW-HFHD pig muscle showed a larger mean diameter of type 1 muscle fibers compared with that of NBF-HF pigs but did not differ with NBW-C and LBW-HF pigs. There was no difference in type IIA and IIB muscle fiber characteristics due to treatment.

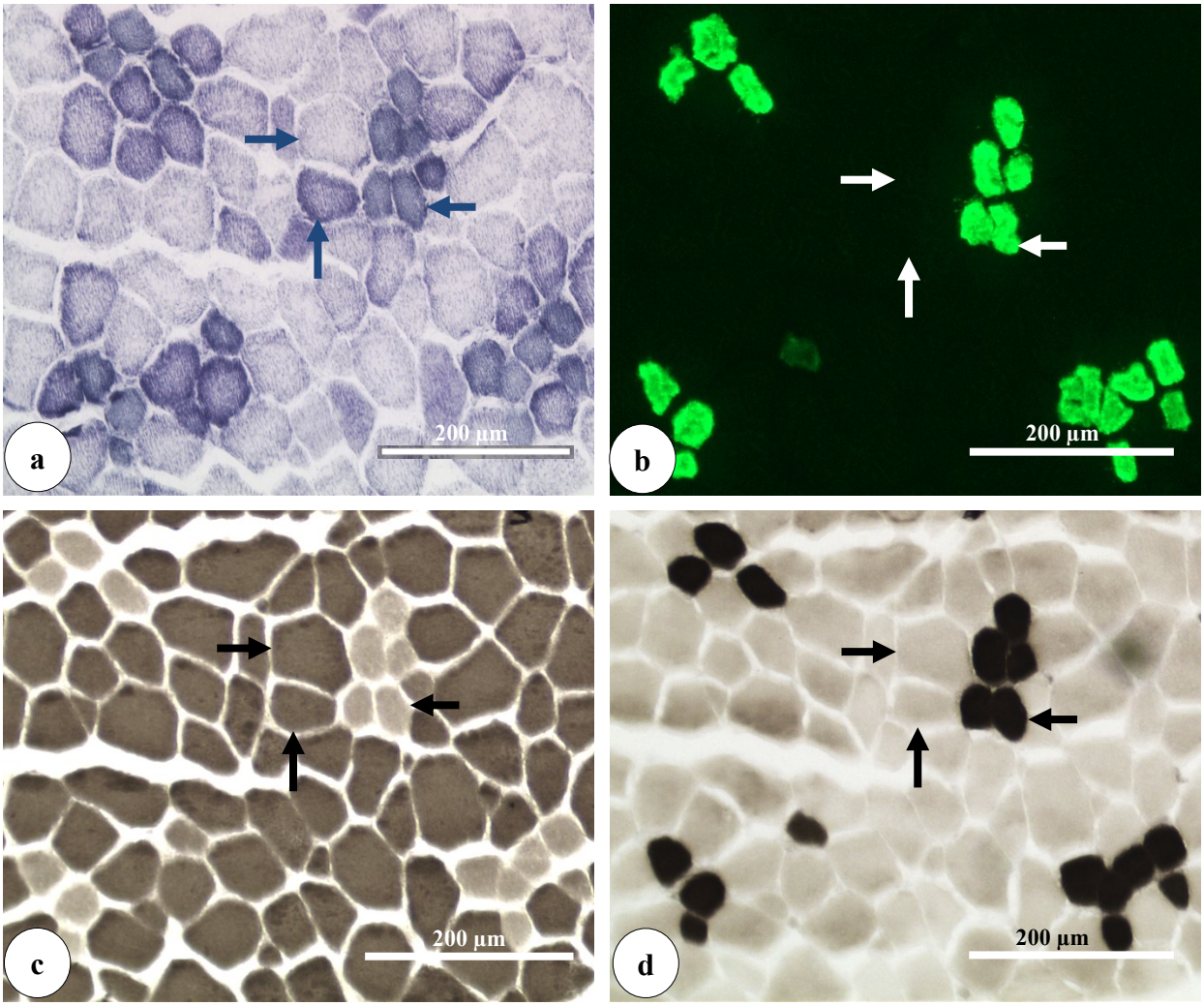

Fig. 2. Histochemistry and immunohistochemistry of muscle fibers typing in muscle serial sections from *Longissimus thoracis* of pork (a) NADH-TR; (b) myosin heavy chain type I primary antibody S58 and Alexa fluor 488 green secondary antibody; (c) myosin ATPase activity after alkaline pre-incubation (pH 10.5) and (d) after acid pre-incubation (pH 4.3). Three types of muscle fibers were identified namely fast-twitch-glycolytic (IIB), fast-twitch-oxidative-glycolytic (IIA) and slow-twitch-oxidative (type I) indicated by right arrow, up arrow and left arrow, respectively. Bars = 200 µm.

Table 5. Effect of different diets and birth weight of piglets on muscle fiber characteristics in *Longissimus thoracis* muscle at 24 h post-mortem

Parameters	NBW-C	NBW-HF	LBW-HF	LBW-HFHD	P-value
n	**5**	**6**	**7**	**5**	
Total muscle fibers in loin muscle area	79152 ± 20129	128875 ± 19125	87888 ±12993	95396 ±20129	0.1073
Type I muscle fibers (%)	10.32 ± 2.10	9.27 ± 2.00	9.66 ± 1.36	6.05 ± 2.10	0.2642
Type IIA muscle fibers (%)	16.40 ± 3.10	19.04 ± 2.95	17.00 ± 2.00	18.03 ± 3.10	0.8436
Type IIB muscle fibers (%)	73.28 ± 3.41	71.69 ± 3.24	73.34 ± 2.20	75.92 ± 3.41	0.6970
Mean muscle fibers diameter (μm)	42.57 ± 2.80b	39.833 ± 2.66b	46.757 ± 1.81ab	51.032 ± 2.80a	0.0057
Type I muscle fibers diameter (μm)	38.86 ± 2.67ab	37.36 ± 2.54b	43.21 ± 1.73ab	44.86 ± 2.67a	0.0401
Type IIA muscle fibers diameter (μm)	38.91 ± 3.06	36.30 ± 2.90	39.38 ± 1.97	40.85 ± 3.06	0.5379
Type IIB muscle fibers diameter (μm)	49.94 ± 3.19	45.85 ± 3.03	51.09 ± 2.06	53.25 ± 3.19	0.1723

Data represents Mean ± standard error (SE)
Different letters (a, b) in the same row are significantly different in different animal groups at the 0.05 level of probability ($P < 0.05$).
NBW = Normal birth weight; LBW = Low birth weight; C = Control (chow diet); HF = High fat diet; HFHD = High fat dairy diet

The frequency distributions of the type I, IIA, IIB and mean muscle fiber diameters across the different class intervals are presented in **Figure. 3**. Type I muscle fiber diameter in the 31-40 μm class interval tended to be more frequent in the NBW pigs than in the LBW-HFHD pigs ($P = 0.0564$). The mean muscle fiber diameter class interval 11-20 μm tended to be significantly more

prevalent for overall (P = 0.01757) and type IIB muscle fibers (P = 0.0211) in the NBW-HF pig muscle compared with that of the LBF-HFHD pigs. Also, the mean muscle fiber with diameters between 61-70 µm tended to be most prevalent (P = 0.0741) in LBW-HFHD pig muscles than in NBW-HF pig muscles.

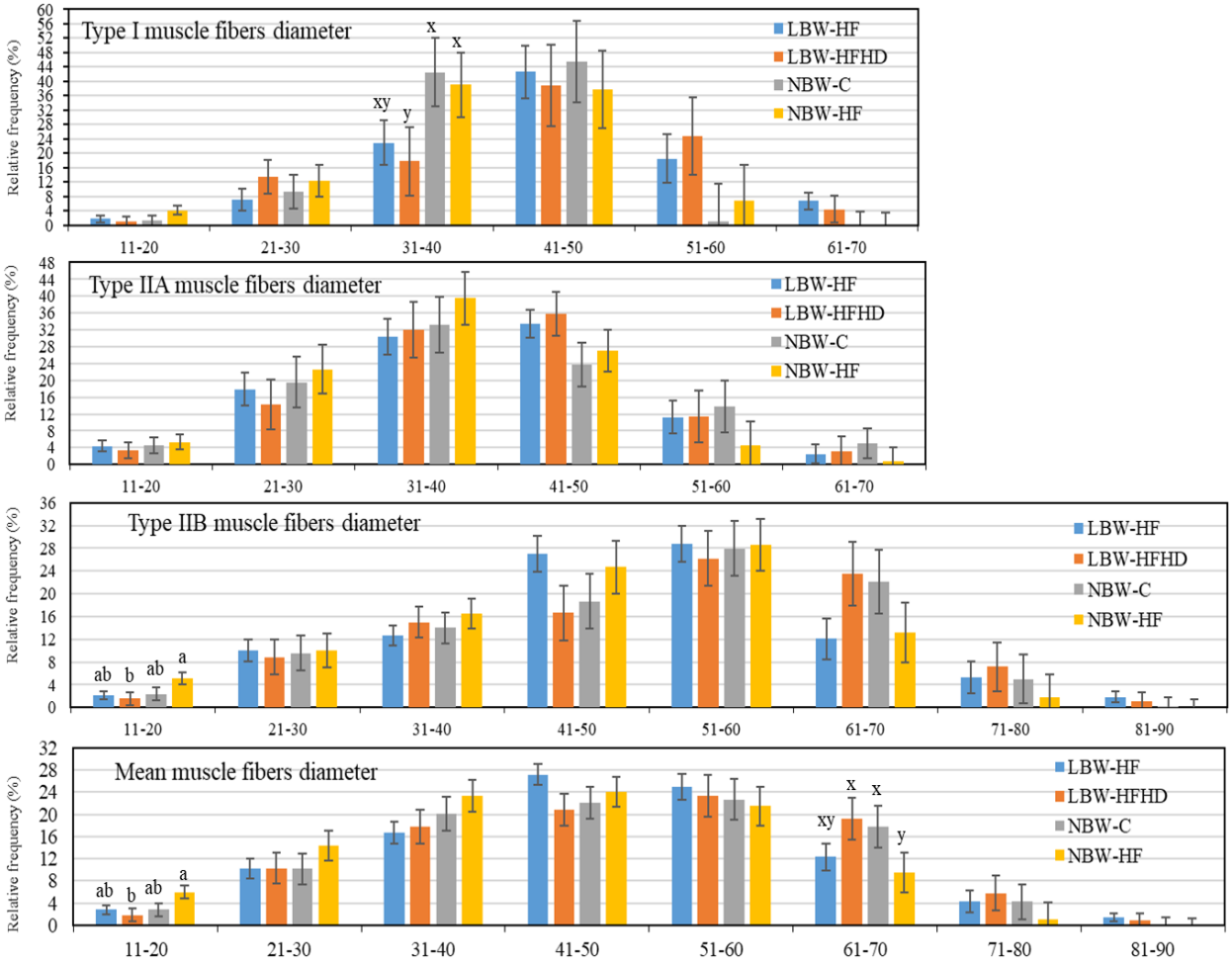

Fig. 3. Frequency distribution of the types I, IIA, IIB and mean muscle fiber diameters for LT muscle from different birth weight groups of pig carcasses. Different letters (a, b) indicate significance (P < 0.05) in same class interval within muscle fiber type and different letters (x, y) indicate differences approached significance (0.08 > P > 0.05) in same class interval within muscle fiber type. Error bars indicate standard error of the mean.

Discussion

The implications of piglet birth weight on subsequent carcass characteristics has been a major subject of pig research in the past (Rehfeldt and Kuhn 2006; Bee et al. 2007; Rehfeldt et al. 2008; Bérard et al. 2010; Alvarenga et al. 2012). The pork industry has increased swine litter size through genetic selection and has been able to introduce highly prolific dam lines for commercial production that has resulted in LBW piglets due to crowding in the uterus of the dam (Quiniou et al. 2002). Also, due to the limited capacity of the uterus, the increased number of fetus results in a reduced nutrient supply per fetus during gestation (Père and Etienne 2000) hence LBW piglets are associated with reduced growth rate during their lifetime (Quiniou et al. 2002; Rehfeldt et al. 2008, Zhang et al. 2018). The results presented in this report disagreed with results from previous studies that showed that lighter piglets at birth (Wolter et al. 2002; Gondret et al. 2005b) or weaning (Mahan and Lepine 1991; Wolter and Ellis 2001; Gondret et al. 2005b) required more days compared to their heavier littermates to attain the same market weight. LBW neonates do not always remain smaller than their littermates throughout their growing period (Crume et al. 2014), and sometimes LBW piglets exhibit catch-up growth postnatally (Douglas et al. 2013; Rutherford et al. 2013; Rehfeldt and Kuhn 2006). Consistent with previous studies (Bérard et al. 2008; 2010), LBW piglets in the present study exhibited compensatory growth as differences in live weight between the different treatments disappeared by 6 weeks of age and LBW-HFHD pigs showed no difference in mean live weights with NBW-C and LBW-HF pigs at weeks 8, 9, 10 and 12.

Gondret et al. (2006) did not observe any significant differences in HCW and dressing percentages between NBW (1.89 ± 0.02 kg) and LBW (1.05 ± 0.04 kg) piglets when the piglets were housed individually, and this was consistent with the results of the present study. Gondret

et al. (2005b) speculated that LBW (0.80-1.10 kg) piglets grow slower than NBW (1.75-2.05 kg) counterparts when placed in collective pens because LBW piglets compete less effectively for feed during the immediate post-weaning period. In this study, the piglets were individually penned and fed hence the LBW piglets had unfettered access to the feed and showed compensatory growth relative to the NBW piglets which accounts for no differences due to diet being observed in HCW and dressing percentages. Other studies (Beaulieu et al. 2010; Gondret et al. 2006) did not observe any differences in the lean meat yield percentages between NBW and LBW pigs, whereas the present study showed that CLY percentage was higher in LBW-HFHD pig carcasses than in those from LBW-HF and NBW-HF pigs but did not differ with NBW-C pigs. Piglet birth weight therefore had no significant effect on Canadian lean yield percentage and loin muscle area in the present study, and this agreed with the results from previous reports (Bee 2004; Bérard et al. 2008).

Heyer et al. (2004) reported that the percentages of primal pork cuts on HCW basis increased, and subcutaneous (back) fat thickness decreased in NBW piglets. The results from the present study contradicted this finding as the subcutaneous fat thickness was significantly lower for carcasses from the LBW-HFHD pigs than those from the LBW-HF and NBW-HF pigs. This may have been associated dairy fat being the major source of energy in the diet. Gondret et al. (2006) observed that shoulder weight percentages did not differ in LBW and NBW piglets but backfat depth and belly percentage were higher in LBW pigs and ham (leg weight) and loin weight were lower in LBW pigs. LBW pigs in previous studies had greater intramuscular fat content as a result of being offered ad-libitum diets during growth (Kuhn et al. 2002; Poore and Fowden 2004) or adjusted daily feed allowance during rearing period (Bee 2004) compared to pigs in the present study. It was reported that this compensatory growth may lead to increase

extra fat deposition in muscle (Ibanez et al. 2011; Cho and Suh 2016) which was absent in the present study. Beaulieu et al. (2010) did not observe any significant effect of piglet birth weight on intramuscular fat content, which agreed with the present study results. There was no significant difference in total intramuscular fat content in the *longissimus* muscle among birth weight groups similar with Gondret et al. (2005b), although they observed a 25 % increase in intramuscular fat content in *semitendinosus* muscle of LBW pigs.

Effects on meat quality were limited and how representative or applicable these results would be at a commercial slaughter weight was not a hypothesis tested in the current study. We did not find any difference in ultimate pH 24h due to treatment, which contradicted with the results of Gondret et al. (2006) who observed higher pH in *longissimus* muscle of LBW piglets compared with NBW piglets but observed no difference in *semitendinosus* muscle. In some previous studies it was observed that birth weight does not have a significant effect on drip loss and cooking loss (Beaulieu et al. 2010; Rekiel et al. 2014) in agreement with the present results although there are contradictory results from Bee (2004) and Gondret et al. (2005a) who found that LBW piglets meat had higher drip loss than NBW piglets. There are reports that demonstrated that LBW piglets have fewer muscle fibers with larger dimeters (Hegarty and Allen 1978; Powell and Aberle 1981; Gondret et el. 2005a) and Lengerken et al. (1997) claimed that meat from these muscles showed increased drip loss.

Beaulieu et al. (2010) observed that birth weight differences of piglets had no significant effect on the color characteristics (L*, a*, b*, chroma and hue values) of the meat, and this result agreed with the present study results. There are other studies that observed increased redness (higher a* values) (Bérard et al. 2008; Rekiel et al. 2014) and increased lightness (higher L* values) when measured at 1-day post mortem (Gondret et al. 2006) in LBW piglets *longissimus*

muscles. The difference in color properties of the pork in the previous studies might be because of the measurement at different post-mortem time. Choi and Oh (2016) observed that pigs with heavier carcass weight had lower redness (a*) values and ultimate pH 24h in *longissimus* muscle compared with light carcass weight pigs and they did not observed difference between these two carcass groups for lightness (L*) and yellowness values. According to these authors, the higher growth rate associated with heavy carcasses produced larger muscle fiber areas in type IIA and IIB muscle fibers and muscles had lower ultimate pH values at 24h postmortem, although it was suggested that increased muscle mass was not always associated with higher glycolytic potential, ultimate pH and meat color (McGilchrist et al. 2016).

In the present study, the loin meat WBSF did not show any significant differences between birth weight groups, which was inconsistent with the results of Gondret et al. (2005b) who concluded that LBW piglets produced less tender meat. Gondret et al. (2005b) found that mean muscle fiber diameter was negatively correlated with tenderness and LBW piglets had larger mean diameter than NBW pigs. In the present study, the WBSF of *longissimus* muscle did not differ between LBW and NBW or dietary fat supplementation, agreeing with the results of Bérard et al. (2008) who did not find any differences in WBSF of cooked *longissimus*.

Differences in muscle fiber characteristics between LBW and NBW pigs were expected. Indeed, previous studies have shown that mean muscle fibers diameter in the *semitendinosus* and *longissimus* muscle of pigs were higher in LBW compared with NBW at the same market BW (Handel and Stickland 1987; Bee 2004; Gondret et al. 2005b) or at the same age (Kuhn et al. 2002). In pig muscle, larger diameter muscle fibers and/or with a reduced total number of muscle fibers has been suggested to lead to a lower pH (Lengerken et al. 1997). The larger diameter or cross-sectional area of muscle fibers in *longissimus* muscle of LBW-HF piglets compared to

NBW agree with findings of other studies (Hegarty and Allen 1978; Powell and Aberle 1981; Gondret et el. 2005a) which found that muscle fibers compensate through increased size and growth. Rehfeldt et al. (2000) suggested that hypertrophy of muscle fibers might compensate for the decrease in the total number of muscle fibers. In the present study, the total number of muscle fibers in the cross-sectional area of *longissimus* muscle did not differ significantly between birth weight treatments, and this result agrees with other studies (Handel and Stickland 1987; Dwyer et al. 1993). There is contradictory evidence of LBW piglets having a lower total number of muscle fibers in *longissimus* muscle compared with NBW piglets (Wigmore and Stickland 1983; Dwyer and Stickland 1991; Rehfeldt et al. 2004; Gondret et al. 2005a). Gondret et al. (2005b) explained that muscle fiber orientation in *longissimus* muscle is not parallel to the longitudinal direction and therefore the estimation of total muscle fibers number is crucial or relevant for this muscle. Also, the skeletal muscle size is determined not just by the number of total muscle fibers it contains, but also by their combined variable cross-sectional area (Dwyer et al. 1993).

It is now an established idea that the number of total muscle fibers in a muscle is fixed before birth in pigs (Wigmore and Stickland 1983) although contradiction exist as Mascarello et al. (1992) reported that the proliferation of muscle fibers occurs also in the neonatal period. The total muscle fiber number in the whole cross-sectional area of *longissimus* muscle was not significantly different due to treatment in the present study. There is evidence that hypertrophy of individual muscle fiber is higher in post-natal period in muscles that have a lower number of muscle fibers (Hegarty and Allen 1978; Rehfeldt et al. 2000). In the present study, the larger mean cross-sectional area of muscle fibers in the LBW-HFHD than in the NBW-HF group at slaughter agrees with the finding of previous reports (Hegarty and Allen 1978; Rehfeldt et al.

2000). It was observed in previous studies (Wigmore and Stickland 1983; Bee 2004; Gondret et al. 2006; Rehfeldt et al. 2008; Beaulieu et al. 2010) that LBW piglets (around 1 kg) muscle had larger mean area for slow-twitch oxidative (type I) muscle fibers similar with the present study. These studies also did not observe any adverse effect on meat quality with increased mean cross-sectional area of type I muscle fibers same with the results of the present study. The result of this study confirmed that skeletal muscle mass is determined by both the total number of muscle fibers and their cross-sectional areas (Dwyer et al. 1993). It has been reported that there is a reduced number of muscle fibers in small pig fetuses (Wigmore and Stickland 1983) but these authors did not confirm that this persisted at slaughter. Moreover, it was reported that muscle fiber type composition is not always correlated with live weight at slaughter hence carcass weight is often used for comparison (Jeong et al. 2012).

In the early post-natal period, muscle fibers are metabolically differentiated into the various types (Maltin et al. 1997; Bee et al. 2007) and are commonly recognized as slow-twitch oxidative (type I), fast-twitch oxidative glycolytic (type IIA), fast-twitch glycolytic (type IIB). Each muscle fiber type differs in metabolism, diameter, and intensity of contraction (Picard et al. 2002). It has been reported that piglet birth weight has no significant effect on muscle fiber type composition at slaughter weight of pigs (Bee 2004; Gondret et al. 2005b; Rehfeldt and Kuhn 2006), and the results of the present study agree. The effect of nutrient deficit during gestation on myogenesis of a fetus is strongly influenced by the time point in gestation at which the deficiency occurs. Nutrient deficiency during the early stages of pregnancy (35-60 days of gestation) hinders the development of primary muscle fibers but deprivation during late gestation (55-90 days of gestation) (Lefaucheur et al. 1995; Kalbe et al. 2017) reduces muscle fiber diameter (Greenwood et al. 1999). Consequently, nutrient deficiency in the early prenatal stage

results in a lower total number of muscle fibers, altered composition of muscle fiber type, and ultimately reduction in overall muscle mass (Ward and Stickland 1991; Yates et al. 2012). During myogenesis, nutrient deficient, growth-restricted fetuses have reduced primary muscle fiber diameter (Wigmore and Stickland 1983). The smaller diameter of primary muscle fibers presents a smaller surface area to serve as a scaffold for the secondary muscle fibers to attach, thereby limiting the number of viable secondary muscle fibers during myogenesis (Wigmore and Stickland 1983). The reduction in primary muscle fiber numbers not only limits muscle growth, but also hampers formation of secondary fibers, which reduces the percentage of fast-twitch myofibers that will take on a glycolytic phenotype at maturity and results in an increase in more oxidative phenotype in the muscle in LBW neonates (Wank et al. 2000; Lefaucheur et al. 2003). For growth, oxidative muscle fibers exhibit greater rates of protein synthesis than glycolytic muscle fibers (Laurent et al. 1978; Bates and Millward 1983; Kelly et al. 1984) and prefer to store energy as fat.

Gondret et al. (2006) observed increased insoluble collagen and a tendency for higher total collagen content in the *longissimus* muscle of LBW pigs compared to NBW pigs with no difference in collagen heat solubility percentages. Further, these authors did not observe any difference in collagen characteristics in the *semitendinosus* muscle regardless of birth weight. Clelland (2001) however found that *semitendinosus* muscle from LBW piglets in the same litter had a higher proportion of intramuscular connective tissue and Karunaratne et al. (2005) found an increase in collagen content associated with LBW. The present study confirmed that intramuscular collagen content and intramuscular fat were not affected by birth weight, suggesting in view of the literature that the longissimus muscle may be less sensitive to the implications of birth weight than the semitendinosus. It is well established that muscle from

LBW piglets contains more intramuscular fat and less lean muscle than NBW piglets (Bee 2004; Poore and Fowden 2004; Gondret et al. 2005b) which ultimately indicates that LBW piglets are prone to increased non-muscle components in their *semitendinosus* muscle mainly as collagen type I and intramuscular fat at 86 days of gestation (Karunaratne et al. 2005). Intramuscular fat and connective tissue cells are the default differentiation pathway when muscle cells are unable to form for any reason during gestation or the early post-natal period (Kablar et al. 2003). Increased intramuscular fat contents are due to larger mean adipocyte diameter in skeletal muscle of LBW piglets than in NBW piglets (Powell and Aberle 1981) although in the present study there was no observed difference in intramuscular fat amount. When LBW pigs were fed the high fat diet, the intramuscular fat contents increased in LBW pigs compared with NBW pigs (Liu et al. 2014). Other studies indicated that intramuscular fat content) as similar among different birth weight pigs at slaughter (Powell and Aberle 1980; Wolter et al. 2002; Gondret et al. 2005b) and this is consistent with the present study. Compared to NBW piglets, LBW piglets had lower subcutaneous fat depth when slaughtered at the same live weight (Rekiel et al. 2015), which contradicted the present study results, although the LBW-HFHD piglets showed decreased subcutaneous fat depth. Collagen content and intramuscular fat in muscle are important characteristics to the meat industry as an increased amount of collagen may increase meat toughness while increased intramuscular fat may improve meat tenderness (Gondret et al. 2005b; Fang et al. 1999). Discrepancies on the effects of birth weight with previous studies on live weight, carcass parameters, physical properties of meat, chemical composition (proximate and collagen content) probably arise from differences in genotype, birth weight delineation, nutrient composition in the diets, feeding level and fat content and sources of fat in the diet.

The reduced nutrient supply at fetal stage can also negatively impact on the total number of muscle fibers in a muscle, leading to restricted growth of muscle postnatally, which may cause increased fat deposition and poor meat quality in pork (Rehfeldt and Kuhn 2006). Rehfeldt et al. (2008) reported poor meat quality in pork from LBW (0.80-1.23 kg) pigs compared with NBW pigs (1.23-1.53 kg) with respect to pH and drip loss and attributed these differences to the excessive fiber hypertrophy. There are also contradictory results indicating that birth weight has no effect on final eating quality of pork (Bérard et al. 2008; Beaulieu et al. 2010), similar with our results. Some earlier studies showed that an increase in the mean cross-sectional area/ diameter of muscle fibers has a negative influence on meat quality (Carpenter et al. 1963; Karlsson et al. 1993; Maltin et al. 1997) specifically increasing WBSF and drip loss (Minelli et al. 1995; Monin et al. 1999). In the present study, it was observed that type I muscle fiber diameter and mean muscle fiber cross-sectional area were larger in LBW-HFHD than NBW-HF pigs. How the source of dietary fat could cause this is unknown, although dairy fat has a high proportion of saturated fats, which have been linked with high satiety (Kozimor et al. 2013), just as dairy protein has been as well in humans (Andersen et al. 2011). The saturated fats of milk have a high proportion of medium chain triacylglycerols as well (Ruiz-Sala et al. 1996), which are also linked to satiety and lower caloric intake in men (St-Onge et al. 2003). The feed intake of the LBW-HFHD pigs was in fact lower than that of pigs receiving the other diets, confirming that the inclusion of dairy fats in the diet decreased appetite. Satiety control in LBW pigs may serve to decrease the amount of energy derived from the diet, thus reducing carcass fat, and increasing lean meat yield.

Conclusion

The study confirmed that LBW piglets can grow at the same rate as NBW piglets when fed individually and that growth is associated with slow-oxidative (type I) muscle fibers hypertrophy. Because of this ability to compensate, there were limited effects of birth weight on carcass yield, carcass quality, and meat quality. Furthermore, the inclusion of dairy fat as the energy source in the early swine diet effectively limited the accretion of excess subcutaneous fat in LBW pigs, suggesting that this may be an effective strategy for producing LBW pig carcasses with composition similar to that of NBW pigs.

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