Intestinal stearoyl-CoA desaturase-1 (SCD1) is differentially expressed along the intestinal length and is regulated through dietary manipulation.

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ABSTRACT OF THE THESIS

Intestinal stearoyl-CoA desaturase-1 (SCD1) is differentially expressed along the intestinal length and is regulated through dietary manipulation

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Stearoyl-CoA desaturase (SCD) is an enzyme that catalyzes the formation of monounsaturated fatty acids from saturated fatty acids. Primarily, the enzyme converts palmitoyl-CoA and stearoyl-CoA to palmitoleoyl-CoA and oleoyl-CoA, respectively. Previous studies have shown that this enzyme is transcriptionally regulated in numerous metabolically-important tissues, such as liver and adipose, in response to nutritional manipulation. However, virtually nothing is known regarding its potential expression and regulation in the intestine, the primary site of lipid absorption in the body. In this study, we aimed to identify whether SCD1 is expressed in the intestine and whether it is regulated in a manner similar to a lipogenic tissue such as liver. Age-matched male wild type C57BL/6J were fasted for 24 hours and then refed a lipogenic high-sucrose very low fat diet (HSVLF) for 16 hours, a paradigm that is known to transcriptionally activate *Scd1* in the liver. Mucosal scrapings were collected from duodenum, proximal jejunum, distal jejunum, ileum and colon. Results show that this refeeding protocol increases SCD1 mRNA and protein expression in duodenum, proximal jejunum, distal jejunum, and ileum. This was also true for several other lipogenic genes, such as sterol regulatory element binding protein (*Srebp-1c*), acetyl-CoA carboxylase (*Acc*), and fatty acid

synthase (*Fas*). SCD1 was expressed at the highest level in the ileum in comparison to duodenum and both proximal and distal sections of jejunum. Immunohistological analyses of Swiss-rolled sections of small intestine showed an increase in SCD1 protein expression after refeeding as well differential SCD1 expression along the length of the intestine, corroborating gene expression data. We conclude that SCD1 is differentially expressed throughout the intestine and is regulated through dietary manipulation.

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Chapter 1: Literature Review

Approximately 70% of men and women in the United States are either overweight, with a BMI between 25 and 29.9, or obese, with a BMI of at least 30 or higher[1]. Visceral obesity in particular, in which an individual has an increased waist circumference size, is a metabolic risk factor that increases ones' risk for developing Type 2 Diabetes[2,3]. Metabolic syndrome is a condition in which one has a cluster of conditions that increases risk for developing heart disease, stroke, as well as Type 2 Diabetes and affects approximately 47 million Americans in the United States[3]. Metabolic risk factors include large waist circumference size, increased plasma triglycerides, decreased plasma high-density lipoprotein (HDL) cholesterol, increased fasting plasma glucose, and increase blood pressure[3,4]. One way to tackle this problem is to understand the mechanisms that contribute to excess energy storage so as to devise nutritional and pharmacological approaches to prevent obesity and manage its sequelae.

One genetic factor that may contribute to excess energy storage is the lipid metabolic enzyme, stearoyl-CoA desaturase (SCD). SCD, a delta-9 desaturase, is an iron containing enzyme located within the endoplasmic reticulum (ER) membrane. It spans the ER membrane with four transmembrane domains that are connected by three hydrophilic loops[5,6] (Fig. 1.1). The protein also contains three conserved histidine motifs, two of which face the lumen of the ER; the loop facing the cytoplasm houses the catalytic sites for desaturase activity[5,6]. SCD inserts a double bond at the delta-9 position of saturated fatty acids (SFA), in turn forming monounsaturated fatty acids (MUFA)[5,7,8]. The primary substrates for this enzyme are palmitoyl-CoA and stearoyl-CoA, and the primary products are palmitoleoyl-CoA and oleoyl-CoA, respectively [5,7,9] (Fig. 1.2).

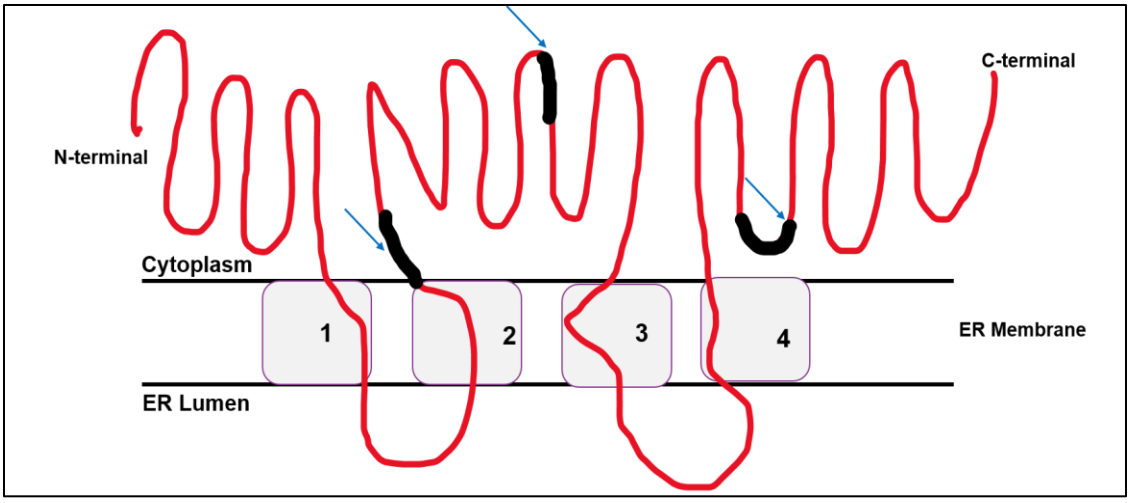


Figure 1.1 Topology of stearoyl-CoA desaturase. Stearoyl-CoA desaturase is located in the endoplasmic reticulum (ER) membrane. It spans the ER membrane with four transmembrane domains, numbered 1-4, connected by 3 hydrophilic loops, drawn in red. Two loops face the ER lumen and one faces the cytoplasm. The one facing the cytoplasm houses two of the three conserved histidine motifs, indicated with blue arrows, which house the primary catalytic sites for desaturase activity.

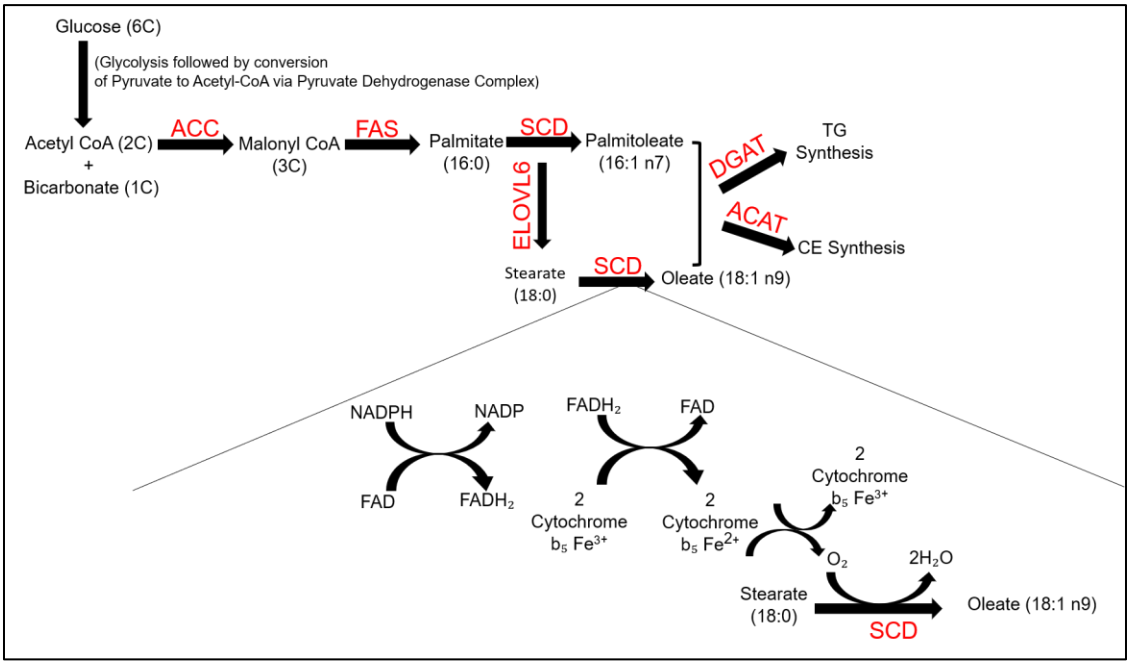


Figure 1.2 Role of stearoyl-CoA desaturase in lipid metabolism. Acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA which is then used to synthesize palmitate (16:0) via the enzyme fatty acid synthase. Palmitate can either undergo desaturation via stearoyl-CoA desaturase or be elongated via fatty acid elongase (ELOVL6) to form stearate (18:0). Subsequently, stearate can be converted into oleate via SCD. *ACAT, acyl-CoA: cholesterol acyltransferase, ACC, acetyl-CoA carboxylase,*

DGAT, diacylglycerol acyltransferase, ELOVL6, fatty acid elongase, FAS, fatty acid synthase, SCD, stearoyl-CoA desaturase.

MUFAs are preferred substrates for subsequent biological reactions such as triglyceride and cholesterol ester synthesis[5,7,9]. There are four isoforms of SCD in mice and two in humans[5,7,10,11]. SCD1 is widely expressed including in liver, adipose tissue, and skeletal muscle, as well as undifferentiated cells of sebaceous glands in skin[5,11–14]. SCD2 is primarily expressed in the brain and plays a major role in embryonic development in mice as mRNA expression of *Scd2* is high between embryonic days 18.5 and 21[14,15]. Targeted deletion of *Scd2* is lethal in the early post-embryonic period[14,15]. SCD2 is also expressed in the liver prior to weaning following which SCD1 expression begins to predominate[14,15]. SCD2 is also expressed in keratinocytes in the skin[14,16]. SCD3 is primarily expressed in the Harderian gland and differentiated sebocytes in skin[5,17]. SCD4 is primarily expressed in the heart[5]. Humans express two SCD isoforms termed hSCD1 and hSCD5. *hSCD1* shares significant sequence homology to mouse *Scd1*[5,10]. *hSCD5*, is expressed primarily in human brain and pancreas[5,7,10]. Unlike *hSCD1*, *hSCD5* has limited homology to mouse isoforms and is a primate specific isoform[5,10,18]. In human studies, the desaturation index, a ratio of MUFA:SFA, is commonly used as an indicator of SCD activity[5,8,19]. Specifically, ratios of 16:1/16:0 and 18:1/18:0 are measured as indicators of SCD activity[5,19]. Palmitoleate (16:1) is primarily endogenously synthesized whereas oleate (18:1) is both endogenously synthesized as well as obtained from the diet[5]. Therefore, the 16:1/16:0 may be a more accurate measure of SCD activity[5]. Higher desaturation ratios, indicative of increased rate of MUFA synthesis by SCD, are directly associated with metabolic syndrome, including diabetes and cardiovascular disease in humans[5,8,19]. An increase in the desaturation

index, corresponding with increased SCD activity, has also been reported to be associated with risk for Alzheimer's disease and colon and esophageal cancers[11,18,20].

SCD1 was initially identified in the 'asebia' mouse that has a naturally occurring mutation in the *Scd1* gene and exhibits alopecia, sebocyte hypoplasia, and resistance to leptin deficiency induced obesity[21–23]. Subsequently, mice with an engineered mutation in the *Scd1* gene were generated to study the role of this enzyme in whole body metabolism[11,24]. Mice with global SCD1 deficiency (*Scd1-/-*) are resistant to high-fat diet (HFD) induced obesity due to significant increases in energy expenditure[9,25]. These mice have reduced hepatic as well as circulating triglycerides and cholesterol esters, dry eye, alopecia, dermatitis, and increased skin barrier permeability[25–27]. To determine the tissue-specific effects of SCD1 deletion, various conditional knockout models have been created and studied. For instance, liver-specific SCD1 deficiency protects mice from carbohydrate-induced *de novo* lipogenesis[9,28]. Adipose-specific deficiency results in decreased inflammation in the adipocyte and increased insulin-independent glucose uptake via upregulation of GLUT1[9,29,30]. Interestingly, the obesity resistant lean metabolic phenotype of global SCD1 deficiency was recapitulated not by liver or adipose-specific SCD1 deletion but by deletion in the skin[31]. Deficiency of SCD1 in the skin resulted in significant increases in energy expenditure and protection from diet-induced obesity, hepatic steatosis, and glucose intolerance[9,13,24,31]. These mice also had an inability to retain heat due to significant alopecia and poor skin integrity resulting from an inability to esterify free fatty acids to cholesterol esters, wax esters, or triglycerides as well as free cholesterol buildup in the skin[9,13,24,31].

The intestine is a vital organ which produces a variety of enzymes as well as hormones to aid the breakdown of carbohydrates, proteins, and lipids. Following the breakdown of these macronutrients, sugars, amino acids, fatty acids and free cholesterol

are absorbed by enterocytes, secreted into the lymphatic and circulatory systems, and delivered to tissues. The primary site of lipid absorption is the jejunum[32]. Lipid digestion, in particular, begins in the stomach where it is mechanically digested with the help of gastric lipase secreted from chief cells in the gastric fundus, making lipid emulsions[32]. These stabilized emulsions are made with phospholipids available from the diet, namely phosphatidylcholine, free fatty acids released from gastric lipase, and free cholesterol that remain on the outer surface of emulsions with triglycerides and cholesterol esters within the core. These emulsions then enter the duodenum for further digestion where bile salts aid in formation of lipid-containing micelles[32]. Pancreatic lipase is secreted from acinar cells from the exocrine pancreas to aid in the breakdown of triglycerides through hydrolysis of the ester bonds at sn-1 and -3, yielding two free fatty acids and one monoacylglycerol (sn-2, 2-MG)[32]. Short and medium chain fatty acids can passively diffuse into the enterocyte, whereas long chain fatty acids require fatty acid transporters and fatty acid translocases such as FAT4 and CD36[32–35]. 2-MG can passively diffuse into the enterocyte but there is also evidence of a protein transporter that may aid in the uptake of 2-MG[35–38]. Once inside the enterocyte, fatty acid binding proteins carry 2-MG and fatty acids to the ER for re-assembly of triglycerides. 2-MG is esterified to another fatty acid via monoacylglycerol acyltransferase-2 (MGAT2) to yield a diacylglycerol[32]. Further, the diacylglycerol is esterified to another fatty acid via diacylglycerol acyltransferase (DGAT) yielding a triacylglycerol[32]. Triacylglycerols are then incorporated into chylomicrons, a primarily triglyceride-rich lipoprotein, that is then transported to the Golgi with the help of microsomal triglyceride transfer protein (MTTP) for subsequent secretion into the lymph where lipids are taken up by extrahepatic tissues first and then finally by the liver[32].

While cholesterol can be synthesized by the liver, it is also obtained from the diet primarily from animal products and dairy products. In the diet, it exists primarily in the non-esterified form. However, 10-15% of the diet contains the esterified form[32]. In the intestine, it can only be absorbed in the non-esterified form[32]. In this case, esterified cholesterol must be hydrolyzed by cholesterol esterase before it is absorbed into the enterocyte[32]. The primary transporter that has been identified in cholesterol transport into the enterocyte is Niemann-Pick C1 like 1 (NPC1L1)[32]. Adenosine triphosphate–binding cassette transporter G5 and G8 (ABCG5 and ABCG8) have also been identified on the apical side as major transporters responsible for cholesterol efflux from the cell into the lumen[32]. Adenosine triphosphate–binding cassette transporter A1 (ABCA1) is the primary transporter on the basolateral side of the enterocyte responsible for cholesterol efflux into circulation for delivery to peripheral tissues through incorporation into HDL. Once inside the cell, it can undergo re-esterification via acyl-CoA:cholesterol acyltransferase (ACAT) where it is esterified to a fatty acid. Following re-esterification, cholesterol esters can also be packaged into chylomicrons in the Golgi for subsequent secretion into the lymph with the help of MTTP[32].

Acyl-CoA:cholesterol acyltransferase 2 (ACAT2) is also an ER membrane enzyme, like SCD1, found in the liver and intestine that catalyzes the synthesis of cholesterol esters from MUFAs and free cholesterol[32,39]. This enzyme lies in close proximity to SCD1 in the ER membrane[13]. This enzyme prefers oleate, one of the products of SCD1, and therefore suggests a potential role of SCD1 in cholesterol esterification in the intestine. Intestine-specific as well as liver-specific deletion of ACAT2 have both been shown to significantly reduce hepatic cholesterol accumulation and provide protection from hypercholesterolemia[39]. Interestingly, skin-specific SCD1 knockouts also exhibit altered handling of cholesterol, in which they have a buildup of

free cholesterol in the skin[31]. However, despite being fed dietary oleate, this phenotype was not rescued in which these mice were not able to utilize oleate either synthesized from other tissues or from the diet to esterify free cholesterol[13]. This suggests that ACAT2 must rely on SCD1-derived oleate to esterify free cholesterol and that cholesterol esterification in the intestine is important for cholesterol absorption. SCD1, in particular, could potentially play a significant role in the cholesterol esterification process in the intestine.

Despite a role for SCD1 in lipid metabolism, virtually nothing is known about a potential role for this enzyme in the intestine and in intestinal lipid absorption or secretion. Before embarking on studies to delineate the functional role of SCD1 in the intestine, we sought to determine if SCD1 was expressed in the intestine, where it was expressed, and if it was regulated by fasting and refeeding a high carbohydrate diet. These studies will form the premise of future studies aimed at understanding the role of intestinal SCD1 in intestinal function and whole-body metabolism.

Chapter 2: SCD1 is differentially expressed along the intestine length and regulated through dietary manipulation

Materials and Methods

Animals and Treatments

Fasting and Refeeding:

Wild-type male C57BL/6J mice (Jackson Laboratories), 14-18 weeks of age, were maintained on a Purina chow maintenance diet (24.7% protein, 13.2% fat, and 62.1% carbohydrate) and individually housed before implementing the diet study. This diet contains 13.2% fat, primarily coming from polyunsaturated fatty acids (PUFA) such as linoleic acid, linolenic acid, arachidonic acid, and omega-3 fatty acids. This diet was not used when refeeding due to a significant amount of PUFAs present within the diet as PUFAs are known to suppress *Scd1* gene expression [40]. For the fasted (F) cohort, male C57BL/6J mice were fasted for 24 hours from 9:00 am to 9:00 am the next day. For the fasted-refed (FRF) cohort, male mice C57BL/6J were fasted for 24 hours from 6:00 pm to 6:00 pm the next day. Mice were then re-fed a high-sucrose very low-fat (HSVLF) diet containing 49% sucrose by weight (20.7% protein, 76.7% carbohydrate, 2.5% fat, 3.53 kcal/g) for 16 hours from 6:00 pm – 9:00 am (Table S1). This diet contains 2.5% fat primarily coming from corn oil, containing linoleic acid as well as traces of linolenic acid, and casein. We chose this fasting-refeeding paradigm as it was previously shown to upregulate hepatic *Scd1*[41]. All mice were euthanized by isoflurane overdose followed by cardiac exsanguination between 9 and 10am and mucosal scrapings were collected for duodenum, proximal jejunum, distal junction, ileum, and colon for gene expression analyses. Duodenum was isolated from pylorus sphincter to the ligament of Treitz. The five centimeters preceding the cecum were denoted as the ileum and tissue extending after the cecum was isolated as the colon. In between, the small intestine was cut in 2 equal parts. The section between the duodenum and ileum was divided into two equal

halves denoted as proximal (close to duodenum) and distal (close to ileum) jejunum. Liver, epididymal adipose tissue (eWAT), and blood were also collected. Tissues were snap-frozen in liquid nitrogen and stored at -80ºC.

Experimental Procedures

Western blotting:

Protein was isolated from livers as well as sections of the small intestine representing duodenum, jejunum, and ileum from age-matched male C57BL/6J mice, 14-18 weeks of age (n=10 for fasted, n=9 for refed), by homogenizing in HEPES buffer containing commercially available protease and phosphatase inhibitors from Thermo Fisher. Samples were centrifuged at 600g for ten minutes at 4˚ C; supernatants were collected and spun again at 600g for ten minutes. The resulting supernatant was collected, protein concentration was quantified using the Bradford assay[42], and aliquots of protein were heated at 70˚ C for ten minutes in commercially available 2x Lamelli Buffer with the addition of 5% 2-mercaptoethanol (BME). Proteins (30ug) were separated by SDS-PAGE and then transferred to a PVDF membrane (Immobilon). After wet transfer, membranes were blocked in 5% BSA TBST overnight at 4˚ C. The following day, membranes were incubated with primary anti-SCD1 monoclonal antibody (Cell Signaling, C12H5) at a dilution of 1:500 at 4˚C overnight. Membranes were then incubated in secondary antibody at a dilution of 1:7000 (HRP-conjugated, anti-rabbit, AC2114, Azure Biosystems) for 1 hour at room temperature before development with enhanced chemiluminescence (Millipore) and imaging on the Azure Biosystems c600 imager. Expression of SCD1 was normalized to either β-actin, GAPDH, or Ponceau.

RNA isolation and Gene Expression:

Mucosal scrapings from age-matched male C57BL/6J mice at 10-13 weeks of age, were collected and homogenized in TriReagent Lysis Buffer for RNA preparation. To make

cDNA, volumes of RNA containing equivalent of 1µg of RNA were used, combining these with nuclease free water from Invitrogen for a total volume of 10µl. Reverse transcription of RNA was facilitated with High Capacity cDNA Reverse Transcription Kit from Thermo Fisher in a total reaction volume of 20µl. cDNA was diluted with 180ul of nuclease free water for gene expression analysis. Analyses of gene expression for genes involved in lipogenesis and were done by real time-qPCR using gene-specific primers. 18s rRNA was used as a housekeeping gene and analysis was carried out using the ΔΔCt method.

Table 1.

Primer List

Gene	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
18S rRNA	TCACCATCATGCAGAACCCA	CCTGGCTGTACTTCCCATCCT
Acc	AGGAGCTGTCTATTCGGGGT	CTGTCCAGCCAGCCAGTATC
Dgat1	CTGGATTGTGGGCCGATTCT	ATACATGAGCACAGCCACCG
Dgat2	GCCTGCAGTGTCATCCTCAT	TGGGCGTGTTCCAGTCAAAT
Fas	TTCCGAGATTCCATCCTACGC	AAAGGTGCTCTCGTCTGTGC
Mogat1	AATCATTGTCCTCGGAGGTG	TGGGTCAAGGCCATCTTAAC
Mogat2	AAGTTCAACGTCCCTGAGGA	AAGTCCCCCTAATCCCACAC
Scd1	CCTCTGGAGCCACAGAACTT	GCCATGGTGTTGGCAATGAT
Scd2	CCACTTGAAAGTAGCCTTAC	ATAGAATAGGGCCACAGCTCA
Srebp-1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT

Table 1. List of forward and reverse sequences qPCR primers

Immunohistochemistry:

The small intestine was divided into two halves. After cutting longitudinally to expose the lumen, each half was rolled into a 'Swiss-roll' from the proximal to distal end with the lumen facing the inside of the Swiss roll. Tissues were fixed in 10% formalin for 24 hours followed by paraffin embedding; serial sections of 5 μm each were obtained by cutting at a depth of 200 μm at the Rutgers Histology Core. SCD1 detection was carried out by using an anti-SCD1 primary antibody. Tissues were deparaffinized using two washes of Xylene and immersion in a graded ethanol series. Antigen retrieval was carried out by immersing slides in boiling citric acid buffer for 20 minutes. Tissues were blocked in 10% normal goat serum for 1 hour at room temperature and incubated in primary antibody (anti-SCD1, Cell Signaling, C12H5) at a concentration of 1:100 overnight at 4ºC. Tissues were then incubated in an anti-rabbit biotinylated secondary antibody followed by ABC reagent for 30 minutes each using a VECTASTAIN Rabbit Elite kit (cat# PK-6101). Negative controls were incubated in 10% normal goat serum during the blocking and primary antibody incubation step, and then with anti-rabbit biotinylated secondary antibody and ABC reagent. Tissues were then incubated in 3,3′-diaminobenzidine (DAB) for visualization. Tissues were dehydrated by immersion in a graded ethanol series and stained with hematoxylin before mounting with non-aqueous mounting media. Images of sections were analyzed using OlyVIA imaging software.

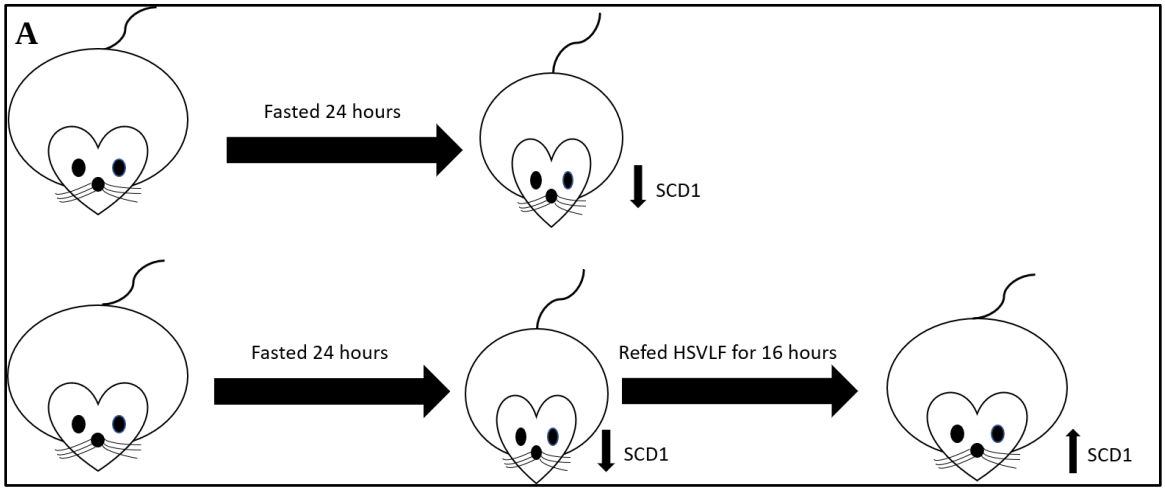
Statistical Analyses

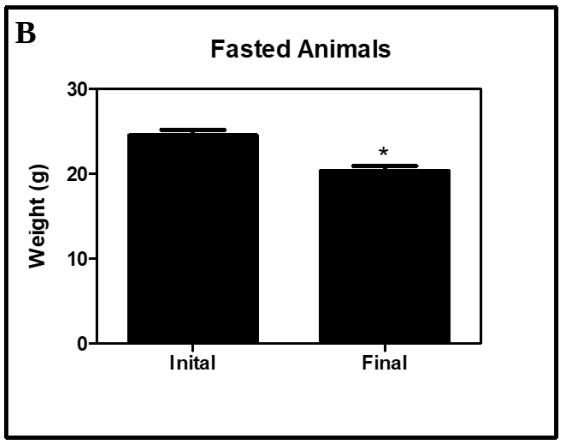
Differences in group means were assessed using two-tailed independent t-tests or one-way ANOVAs followed by Bonferroni correction for multiple comparisons as needed for multi-group comparisons[43,44]. Statistical tests were carried out in GraphPad Prism. Statistical significance was defined as $p < 0.05$.

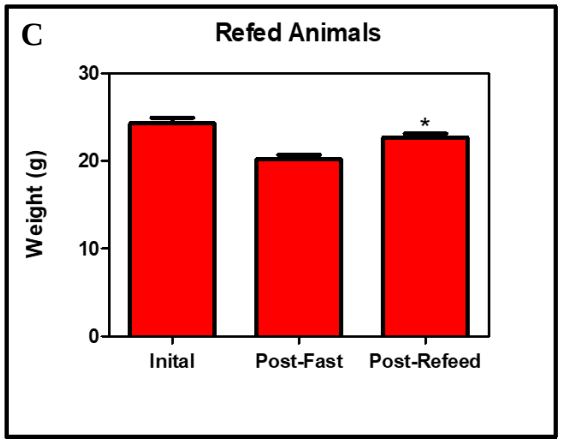
Results

Changes in Body Weight and Food Intake

Mice were fasted and refed as depicted in Fig. 2.1A. A 24-hour fast resulted in approximately 17% loss of initial body weight (Fig. 2.1B). During the refeeding period, animals regained about 13% of their initial body weight and consumed an average of 2.7 grams of the HSVLF diet (Fig. 2.1C-D).







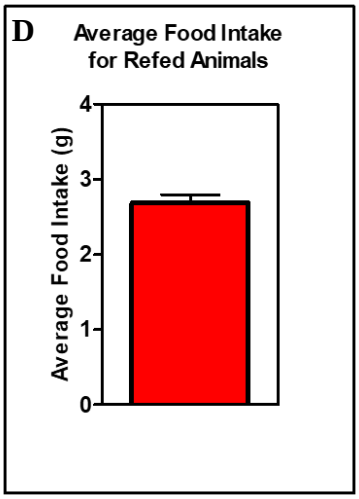


Figure 2.1 Experimental design and changes in body weight and food intake. Male mice (n=10) were fasted for 24 hours. Initial and final body weights were recorded. In the refed cohort, mice (n=10) were fasted for 24 hours, and then refed a HSVLF diet for 16 hours. Initial, fasted, and refed body weights were recorded. Food intake during the refeeding period was also measured. Values are expressed as means ± SEM. *p<0.05 vs. initial body weight using two-tailed paired t-test.

Gene expression of intestinal desaturases

Gene expression of *Scd1* in liver was quantified as a positive control as it is known to be induced by this fasting-refeeding paradigm[41]. Indeed, hepatic *Scd1* was induced by approximately 17.5-fold upon refeeding the HSVLF diet (Fig. 2.2A). *Scd1* expression in adipose tissue is unaffected by this dietary paradigm (Fig. 2.2B), as has been previously shown[45]. *Scd1* expression in the different segments of the small intestine and colon were also induced to varying extents by refeeding the HSVLF diet. In the proximal jejunum, *Scd1* expression increased by approximately 12-fold; distal jejunum 19.4-fold, ileum 20.4-fold, and colon 4-fold (Fig. 2.3B-E). Interestingly, there did not seem to be a significant induction of *Scd1* expression in duodenum (Fig. 2.3A). When comparing all five sections of the intestine, there is a gradient increase in *Scd1* expression along the small intestine with *Scd1* being expressed at the highest level in distal portions of the intestine. Relative to duodenum in the fasted state, *Scd1* was enriched 0.7-fold in

proximal jejunum, 1.8-fold in distal jejunum, 4.2-fold in ileum, and 3.7-fold in colon (Fig. 2.3F). Differences in expression of *Scd1* in fasted animals were found to be statistically significant as determined by one-way ANOVA ($p < 0.05$). *Scd1* expression was significantly higher in ileum in comparison to proximal jejunum ($p<0.05$). Additionally, the increase in *Scd1* expression in the colon was significantly higher than that seen in proximal jejunum ($p < 0.05$). Relative to duodenum in the refed state, *Scd1* was enriched 4-fold in proximal jejunum, 8.3-fold in distal jejunum, 19.5-fold in ileum, and 7.9-fold in colon (Fig. 2.3G). Differences in expression of *Scd1* in refed animals were found to be statistically significant as determined by one-way ANOVA ($p < 0.05$). *Scd1* expression is significantly higher in distal portions of the intestine in comparison to proximal portions. In particular, *Scd1* expression in distal jejunum, ileum, and colon was significantly higher relative to duodenum ($p < 0.05$). *Scd1* expression in ileum was significantly higher relative to proximal jejunum ($p < 0.05$) and distal jejunum ($p < 0.05$). *Scd1* expression decreased in the colon relative to ileum ($p < 0.05$). Although the primary site of lipid absorption is in jejunum, the highest level of *Scd1* expression in distal portions of the intestine, such as ileum, suggest a relatively unexplored physiological importance of this protein in distal portions of the small intestine.

Scd2 is primarily expressed within the brain, however during the course of these studies, we discovered that in addition to *Scd1*, *Scd2* was also expressed along the length of the intestine and was also upregulated in a manner similar to *Scd1* (Fig. 2.4A-G). Interestingly, unlike *Scd1*, *Scd2* was induced 14.5-fold in the duodenum (Fig. 2.4A), upon refeeding. In the proximal jejunum, *Scd2* expression increased by 27.9-fold; distal jejunum 52.5-fold, ileum 29.8-fold, and colon 5.8-fold (Fig. 2.4B-E). Like *Scd1*, there was also a gradient increase of *Scd2* along the small intestine with *Scd2* being expressed the highest in distal portions of the intestine. Relative to duodenum in the fasted state, *Scd2* was enriched by 0.8-fold in proximal jejunum, 2.6-fold in distal jejunum, 5.2-fold in ileum,

and 11.7-fold in colon (Fig. 2.4F). Differences in expression of *Scd2* in fasted animals were found to be statistically significant as determined by one-way ANOVA ($p < 0.05$). *Scd2* expression was significantly higher in colon relative to duodenum ($p < 0.05$), proximal jejunum ($p < 0.05$), distal jejunum ($p < 0.05$), and ileum ($p < 0.05$). Relative to duodenum in the refed state, *Scd2* was enriched by 2.3-fold in the proximal jejunum, 11.5-fold in distal jejunum, 16.3-fold in ileum, and 6.7-fold in colon (Fig. 2.4G). Differences in expression of *Scd2* in refed animals were found to be statistically significant as determined by one-way ANOVA ($p < 0.05$). Specifically, *Scd2* expression was significantly higher in ileum relative to proximal jejunum ($p<0.05$) and duodenum ($p<0.05$).

When comparing relative expression of *Scd1* vs. *Scd2* within each section of the intestine, *Scd2* expression was consistently higher than *Scd1*. Relative to *Scd1* expression in refed animals, *Scd2* expression was 27.3-fold higher in duodenum, 14.8-fold higher in proximal jejunum, 23-fold higher in distal jejunum, 12.2-fold higher in ileum, and 19.6-fold higher in colon (Fig. 2.5A-E).

Our discovery of *Scd2* expression in the intestine raises many questions about the relative contributions of two different SCD isoforms in the same tissue. Unlike *Scd1*, *Scd2* was shown to be upregulated by 15-fold in duodenum after refeeding (Fig. 2.4A).

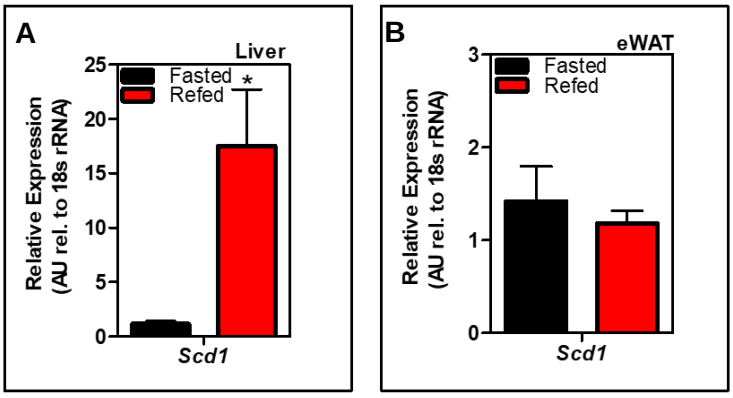


Figure 2.2 Expression of *Scd1* in lipogenic tissues, such as liver, is upregulated. RNA was isolated from individual liver and eWAT samples (n=5 per group). Gene expression shows *Scd1* is highly regulated in liver (A). Values expressed as means ± SEM. *p<0.05 vs. fasted using two-tailed independent t-test. *Scd1, stearoyl-CoA desaturase-1*.

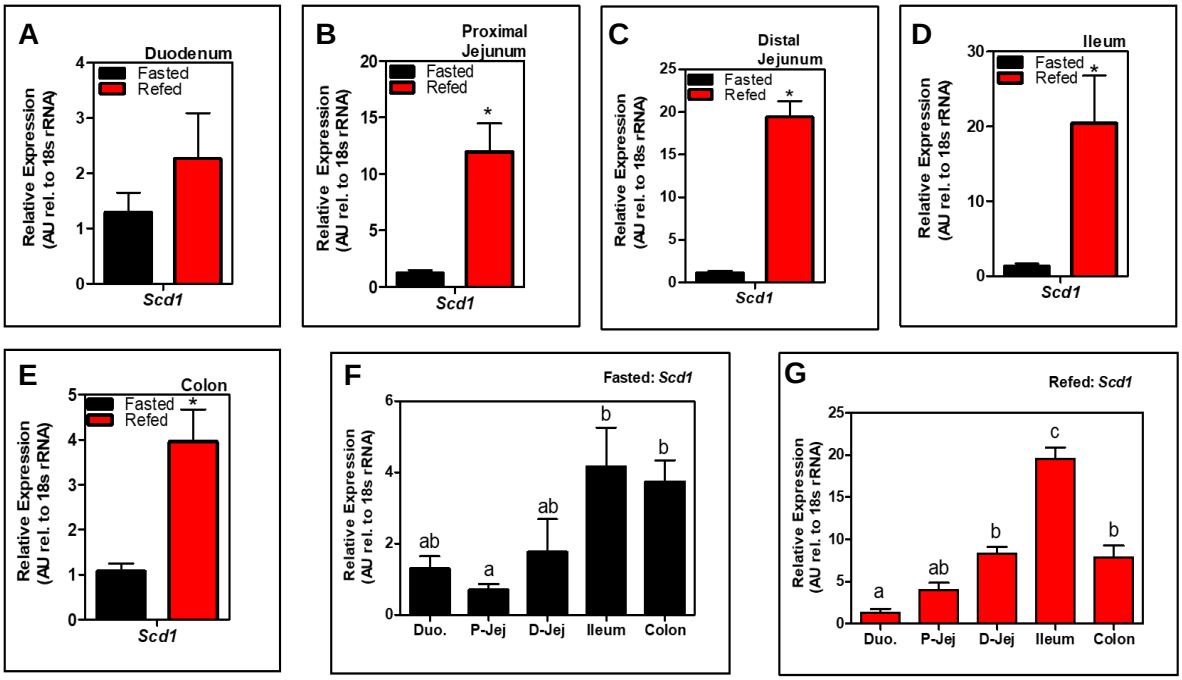


Figure 2.3 *Scd1* is upregulated in the small intestine after refeeding and is expressed the highest in distal portions of the intestine. Mucosal scrapings were collected from each segment of the small and large intestine (n=8 per group). *Scd1* was found to upregulated after refeeding in proximal jejunum, distal jejunum, ileum, and colon (B-E). Little induction of *Scd1* was observed in duodenum (A). *Scd1* was expressed highest in distal portions of the intestine, including distal jejunum, ileum and colon (F and G). Values expressed as means ± SEM. *p<0.05 vs. fasted using two-tailed independent t-test for figures A-E. For figures F and G, a one-way ANOVA followed by Bonferroni correction was conducted to determine statistical significance between multiple groups. Bars with different letters are statistically different from each other (p<0.05, F and G). *Scd1, stearoyl-CoA desaturase-1.*

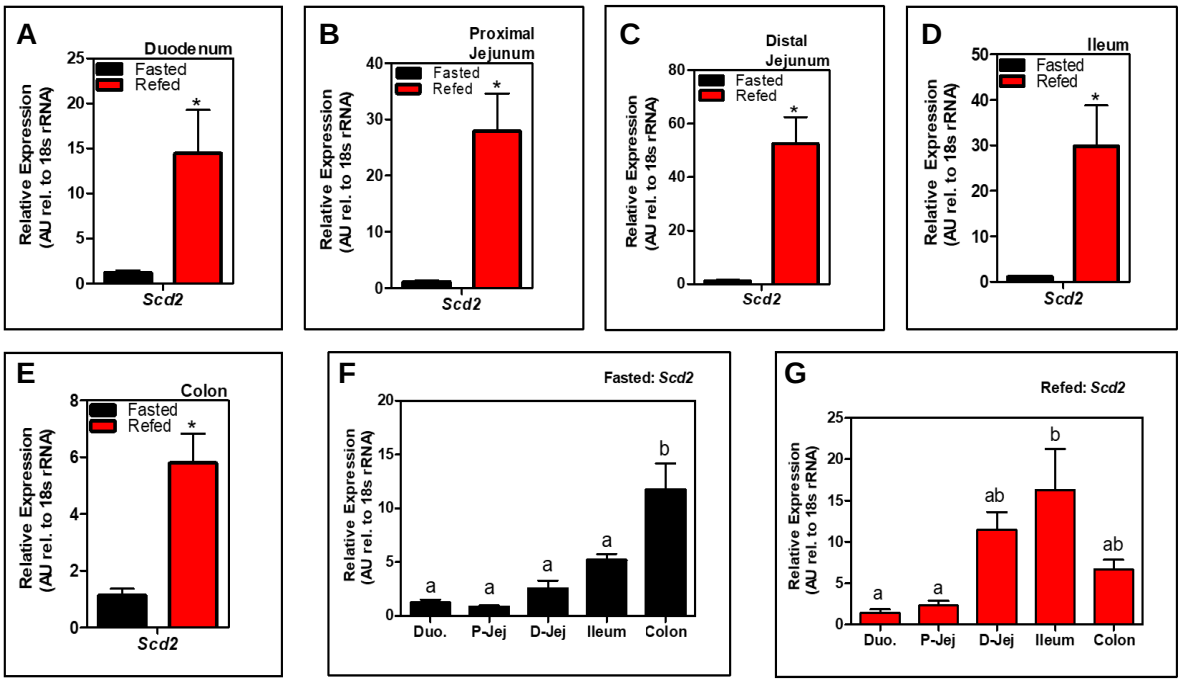


Figure 2.4 *Scd2* is upregulated after refeeding in the intestine and is most highly expressed in distal portions of the intestine. Mucosal scrapings were collected from each segment of the small and large intestine (n=8 per group). *Scd2* was found to be upregulated similar to *Scd1* in all tissues except duodenum (A-E). *Scd2* was expressed highest in distal portions of the intestine including ileum and colon (F and G). Values expressed as means ± SEM. *p<0.05 vs. fasted using two-tailed independent t-test for figures A-E. For figures F and G, a one-way ANOVA followed by Bonferroni correction was conducted to determine statistical significance between multiple groups. Bars with different letters are statistically different from each other (p<0.05, F and G). *Scd2, stearoyl-CoA desaturase-2.*

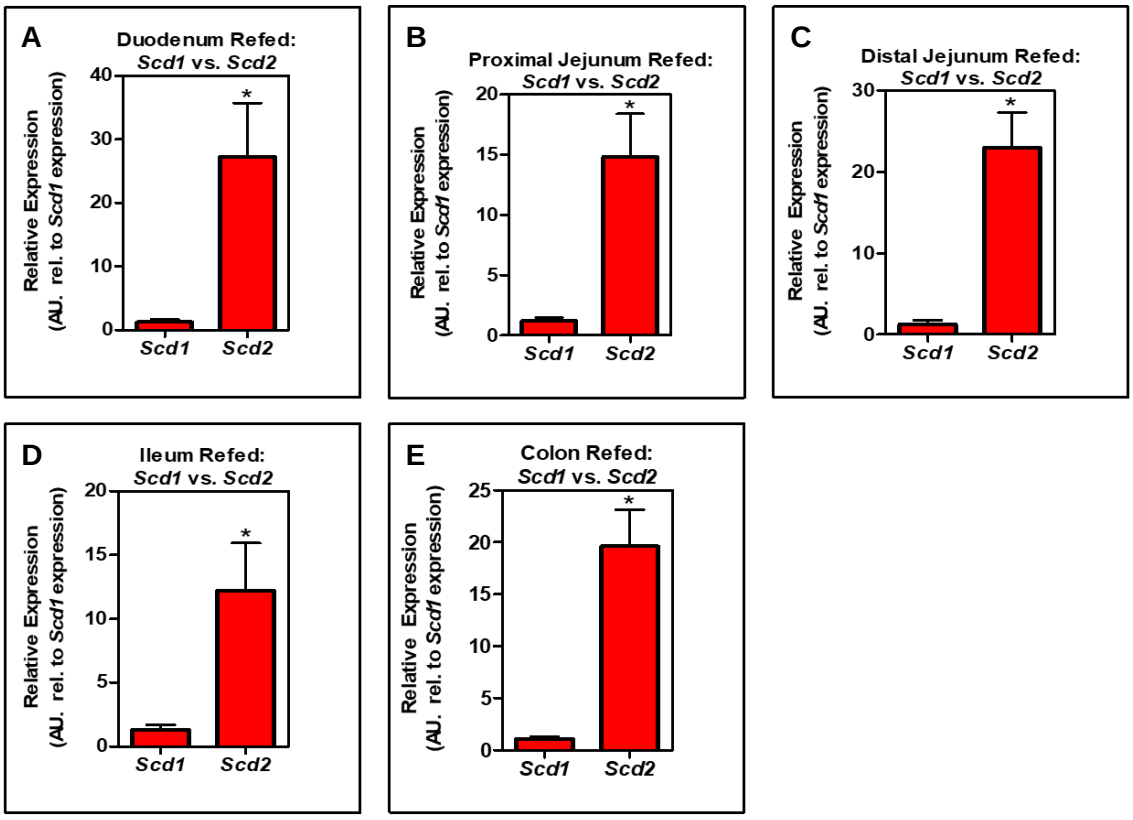


Figure 2.5 Relative expression of *Scd1* vs. *Scd2* in the intestine after refeeding HSVLF diet. Mucosal scrapings were collected from each segment of the small and large intestine (n=8 per group). *Scd2* was found to be expressed higher than *Scd1* in all parts of the intestine (A-E). Gene expression of *Scd2* was quantified relative to *Scd1* expression as control. Values expressed as means ± SEM. *p<0.05 vs *Scd1* using two-tailed independent t-test. *Scd1, stearoyl-CoA desaturase-1, Scd2, stearoyl-CoA desaturase-2.*

Expression of other lipid metabolic genes in the small intestine

In order to determine if other genes regulating lipid synthesis and metabolism are regulated in a manner similar to *Scd1,* we measured gene expression of *Acc*, *Fas*, *Srebp-1c*, *Mgat1*, *Mgat2*, *Dgat1,* and *Dgat2* within the same mucosal scrapings along with liver and eWAT as positive controls. In liver, the primary genes that were upregulated after refeeding were *Acc* by 14.4-fold, *Fas by* 68.2-fold, and *Srebp-1c* by 49.7-fold (Fig. 2.6A). In eWAT, the primary genes that were upregulated the most were *Fas* by 19.4-fold and *Dgat2* by 11.6-fold (Fig. 2.6B). The primary genes that were

upregulated in the small intestine were *Acc*, *Fas*, and *Srebp-1c* (Fig. 2.7A-D). *Acc* was significantly upregulated by 2.3-fold in proximal jejunum, 2.2-fold in distal jejunum, and 3.3-fold in ileum (Fig. 2.7B-D). *Fas* was significantly upregulated by 3.4-fold in duodenum, 2.5-fold in proximal jejunum, 3.2-fold in distal jejunum, and 2.8-fold in ileum (Fig. 2.7A-D). *Srebp-1c* was significantly upregulated by 3.5-fold in duodenum, 6.1-fold in proximal jejunum, 10.5-fold in distal jejunum, 16.5-fold in ileum, and 7.8-fold in colon (Fig.2.7A-E). *Mgat2* was significantly upregulated by 2.2-fold in proximal jejunum, and 1.9-fold in distal jejunum (Fig. 2.7B-C). There were no statistically significant changes in expression of *Dgat1* within the small intestine. *Dgat2* was significantly upregulated in distal jejunum by 1.9-fold (Fig. 2.7C). An increase in *Srebp-1c*, a transcription factor that facilitates the upregulation of genes involved in lipogenesis, could be indicative of a *Srebp-1c*-dependent upregulation of lipogenesis, as *Acc*, *Fas*, and *Scd1* are all known target genes of SREBP-1c. SREBP-1c itself regulates its own transcription. Although there is a tendency towards an increase in most lipogenic genes within the colon, these were not statistically significant. *Srebp-1c*, *Mgat2*, and *Dgat2* seemed to be the most significant in this case (Fig 2.7E). In terms of *de novo* lipogenesis ACC synthesizes malonyl-CoA from acetyl-CoA and bicarbonate. FAS then uses malonyl-CoA to add carbons primarily synthesize palmitoyl-CoA (16:0). Palmitoyl-CoA can then either undergo desaturation at the delta 9 position via SCD1 or be elongated to stearoyl-CoA (18:0) via a long-chain fatty acid elongase (Elovl6). 18:0 can then undergo desaturation via SCD1 to form oleoyl-CoA (18:1). MUFAs are then esterified to a monoacylglycerol to yield diacylglycerol via MGAT. Subsequently DGAT will then esterify another MUFA to diacylglycerol to make triacylglycerol. While there is an overall increase in genes involved in *de novo* lipogenesis, their overall physiological role is unclear. The intestine is not considered a lipogenic organ. Therefore, it is possible that regulation of these

genes serves to generate lipids that may play a regulatory role in the enterocyte, as discussed further in the Discussion section.

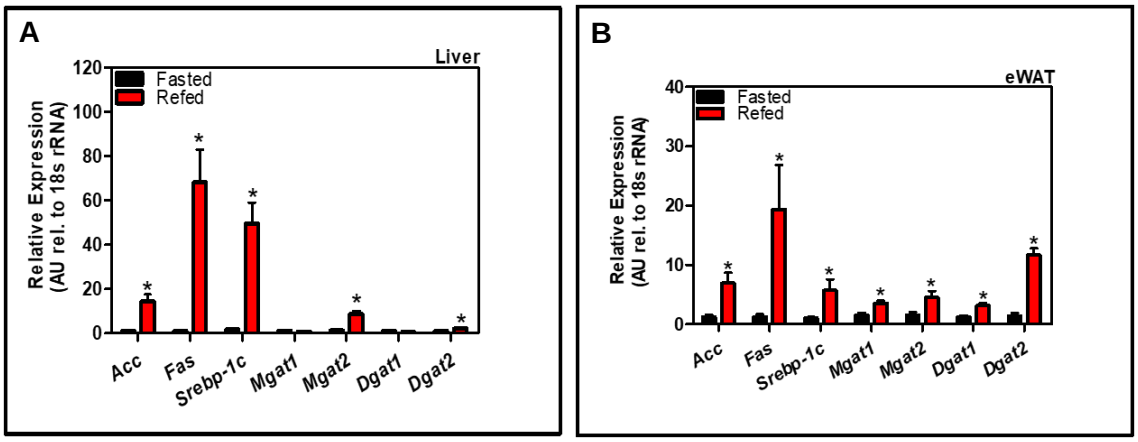
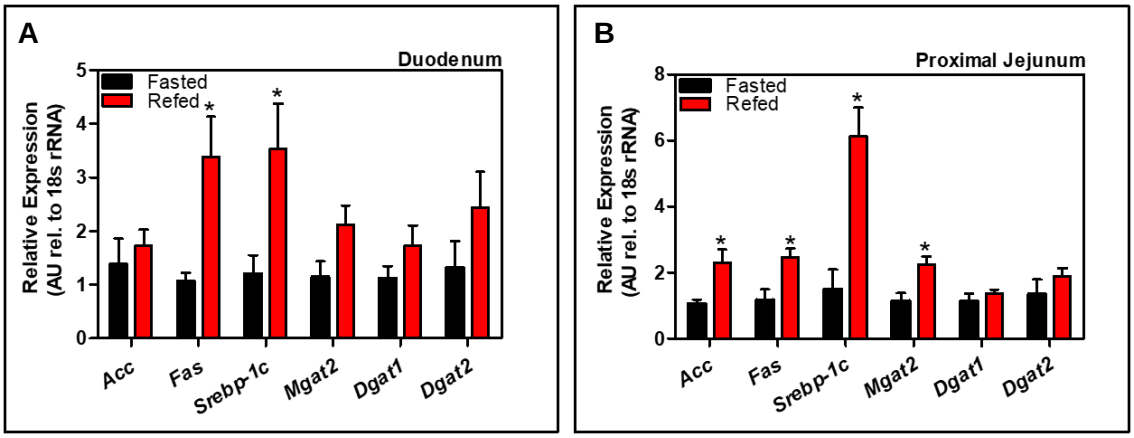


Figure 2.6 mRNA expression of lipid metabolic genes increase after refeeding in lipogenic tissues such as liver and epididymal adipose tissue. Expression of *Acc, Fas, Srebp-1c, Mgat1, Mgat2, Dgat1, and Dgat2* were quantified relative to 18s rRNA (n=5 per group). There is an overall increase in lipogenic genes within liver as well as eWAT. Values expressed as means ± SEM. *p<0.05 vs. fasted using two-tailed independent t-test. *Acc, acetyl-CoA carboxylase, Dgat1, diacylglycerol acyltransferase 1, Dgat2, diacylglycerol acyltransferase 2, Fas, fatty acid synthase, Mgat1, monoacylglycerol acyltransferase 1, Mgat2, monoacylglycerol acyltransferase 2, Srebp-1c, sterol regulatory element-binding protein.*



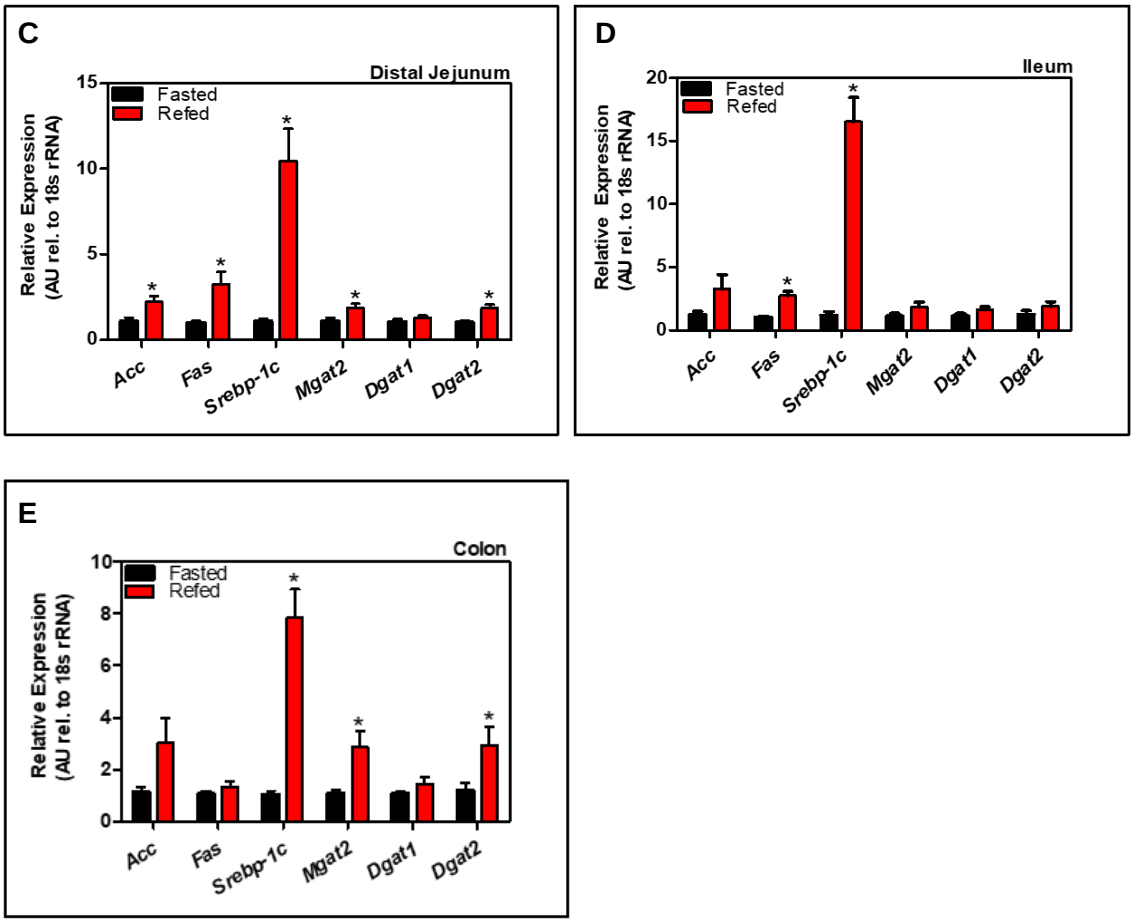


Figure 2.7 mRNA expression of lipid metabolic genes increase after refeeding in the intestine. Expression of *Acc, Fas, Srebp-1c, Mgat2, Dgat1, and Dgat2* were quantified relative to 18s rRNA (n=8 per group). Similar to *Scd1* and *Scd2*, there is an overall increase in lipogenic genes within the small intestine as well as large intestine. Liver and eWAT were used as positive control. Values expressed as means ± SEM. *p<0.05 vs. fasted using two-tailed independent t-test. *Acc, acetyl-CoA carboxylase, Dgat1, diacylglycerol acyltransferase 1, Dgat2, diacylglycerol acyltransferase 2, Fas, fatty acid synthase, Mgat2, monoacylglycerol acyltransferase 2, Srebp-1c, sterol regulatory element-binding protein.*

Protein Expression- Western Blotting and IHC

To determine if gene expression translates to protein expression, whole cell tissue lysates were prepared and used to evaluate protein expression by Western blotting. Liver lysates were used as positive controls for induction of SCD1 by fasting-refeeding (Fig. 2.8A). As seen with gene expression, SCD1 protein increased with refeeding in jejunum and ileum (Fig. 2.8A). SCD1 expression in duodenum is relatively low,

consistent with gene expression results. Indeed, consistent with transcriptional regulation of *Scd1*, SCD1 protein was expressed at the highest level in the ileum and distal jejunum, with lower level in the proximal jejunum, and the lowest levels in duodenum (Fig. 2.8B). This pattern of SCD1 expression was also corroborated by IHC in the refed state with increased and darker staining of SCD1 in liver as positive control (Fig. 2.9A). An increase and darker staining of SCD1 was also observed after refeeding in all sections of the small intestine (Fig. 2.10A-D). SCD1 staining showed an increase in concentration and intensity along the small intestine with darker staining in the distal small intestine in comparison to the proximal small intestine (Fig. 2.11A).

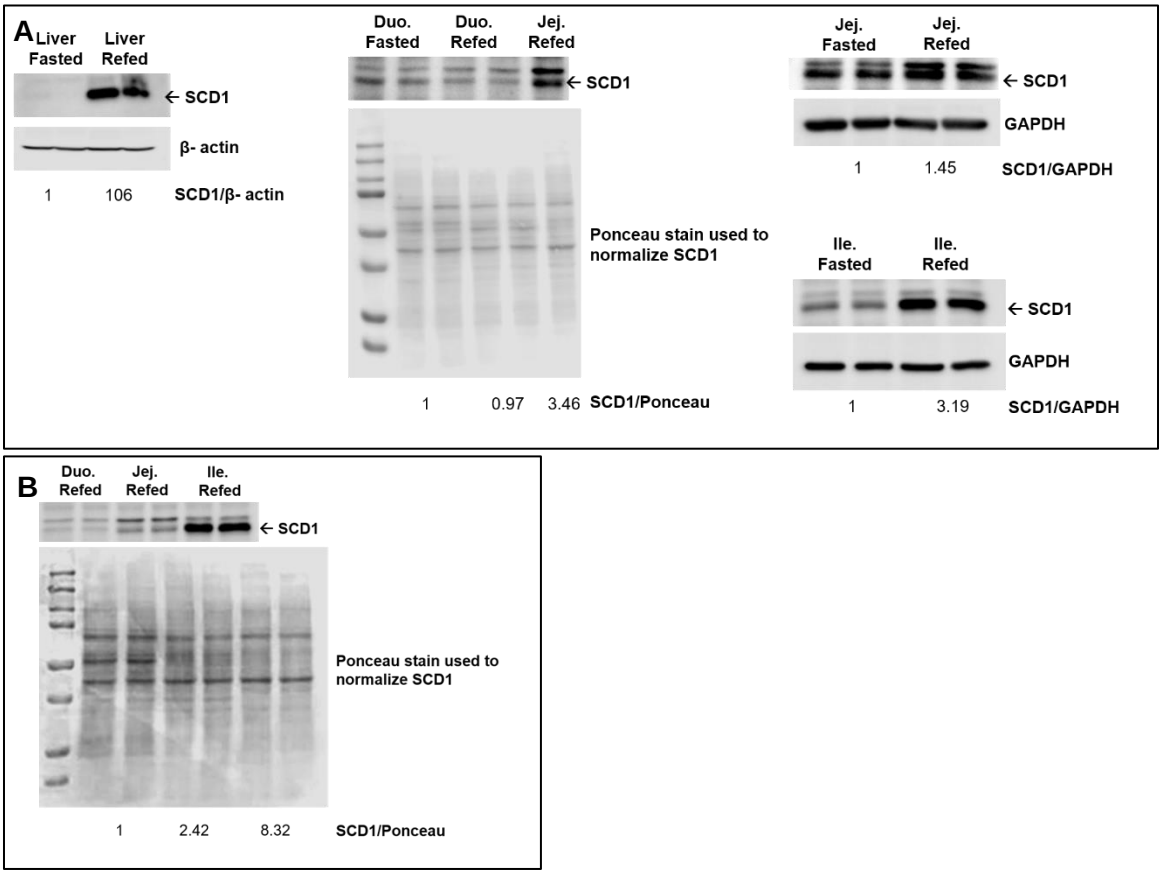


Figure 2.8 Protein expression of SCD1 in duodenum, jejunum, and ileum increases after refeeding. Western blot analysis of SCD1 for pooled fasted samples (n=10) and refed samples (n=9) shows an increase in SCD1 expression after refeeding in jejunum and ileum (A). All tissues were normalized using either B-actin, GAPDH, or Ponceau as control. SCD1 is expressed at the highest level in the ileum (B). Similar to gene

expression, SCD1 protein expression is highest in ileum. *GAPDH, Glyceraldehyde 3-phosphate dehydrogenase, SCD1, stearoyl-CoA desaturase-1.*

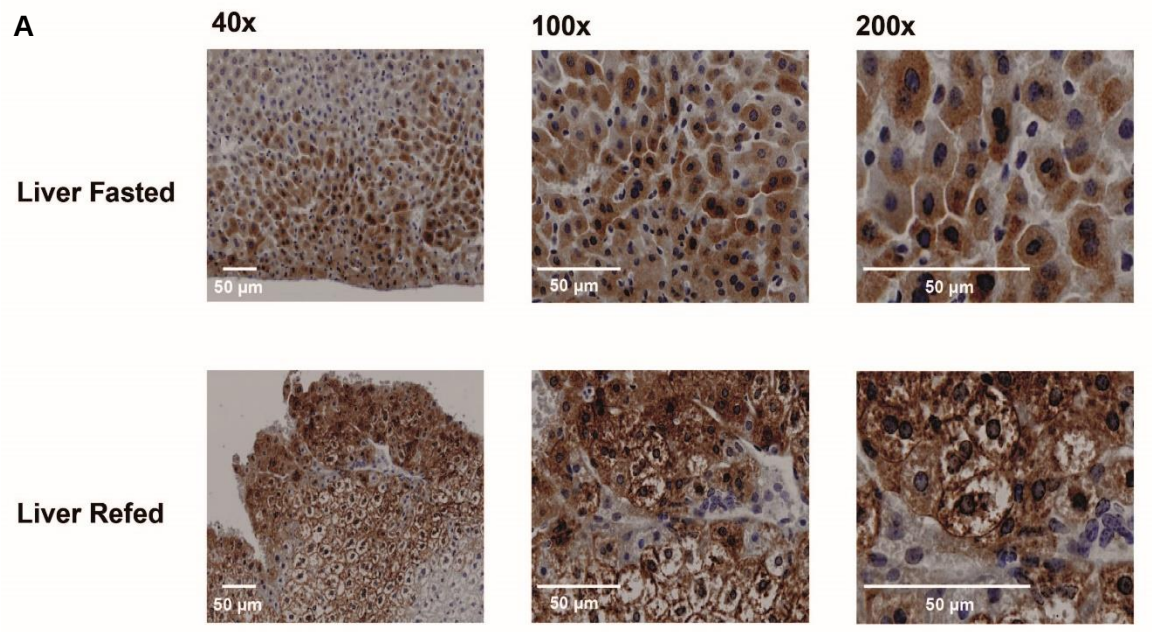
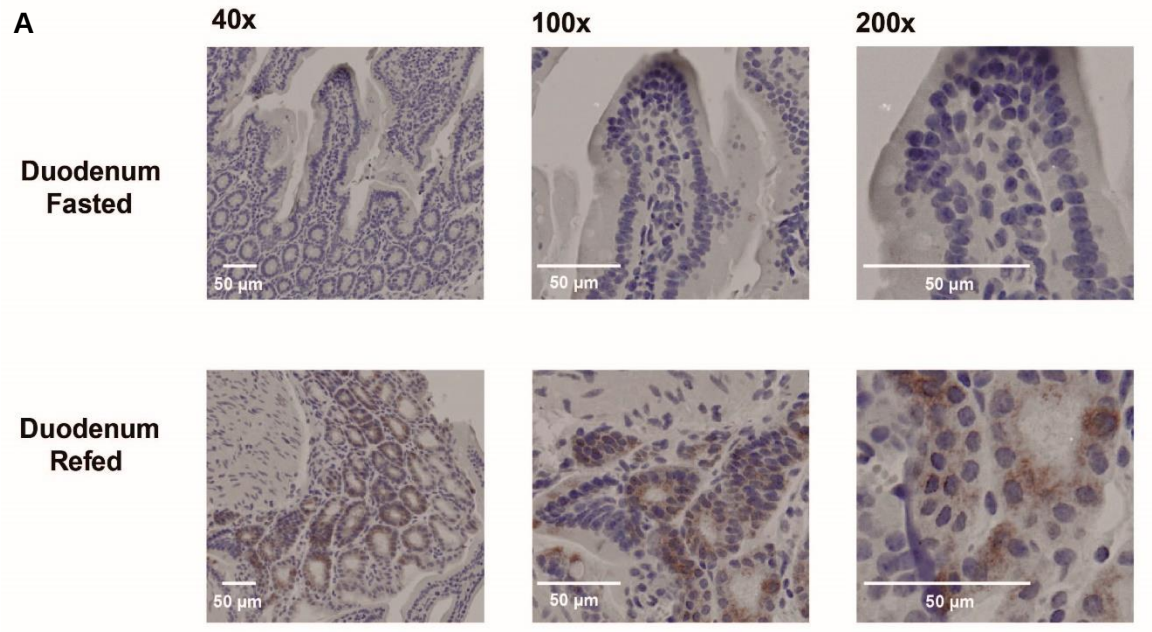
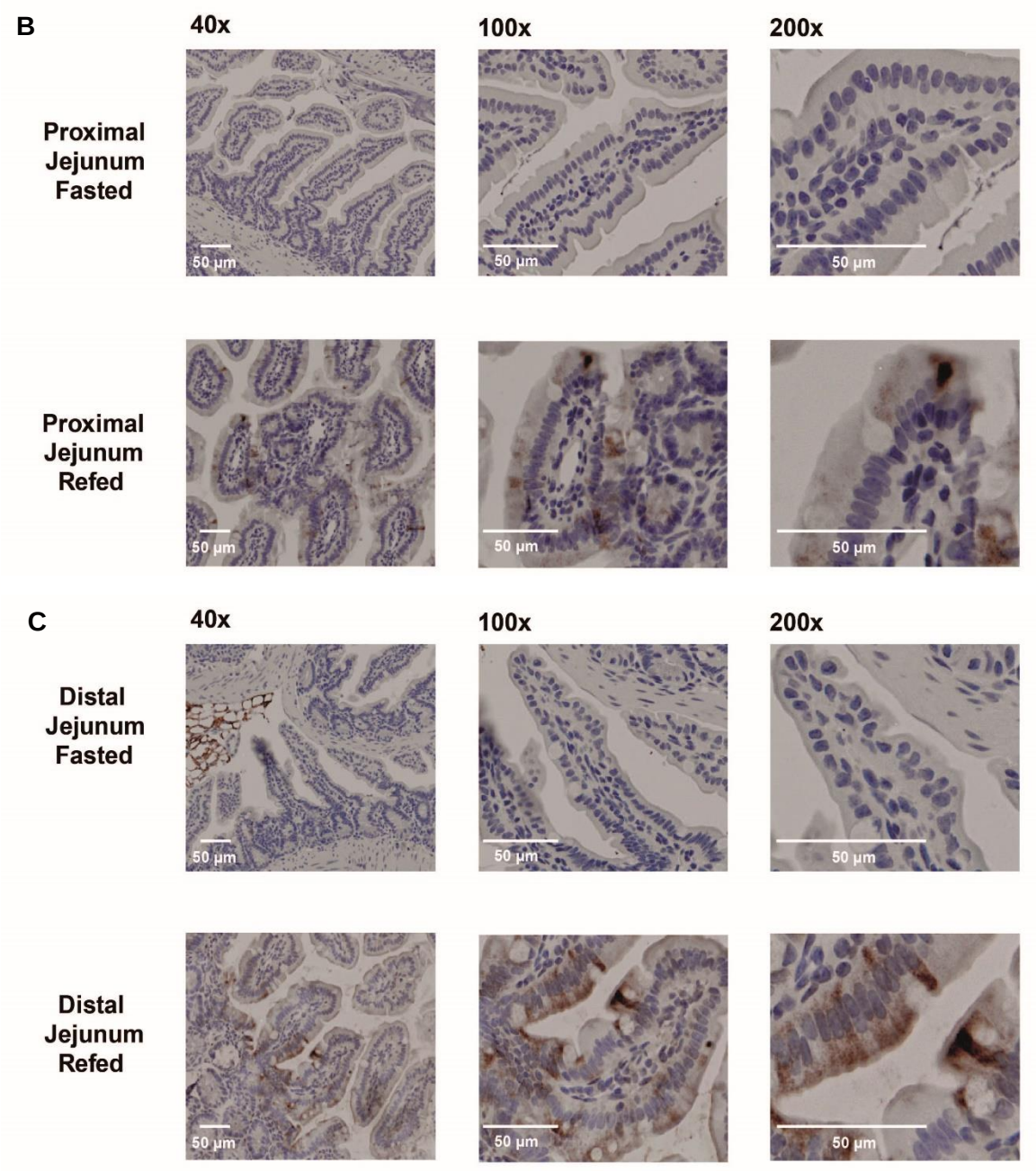


Figure 2.9 SCD1 in liver fasted and refed samples increases after refeeding, as positive control. Increased staining for SCD1 in liver after refeeding a HSVLF diet (n=5 per group).




B
40x
100x
200x
Proximal Jejunum Fasted
Proximal Jejunum Refed
C
40x
100x
200x
Distal Jejunum Fasted
Distal Jejunum Refed
50 µm

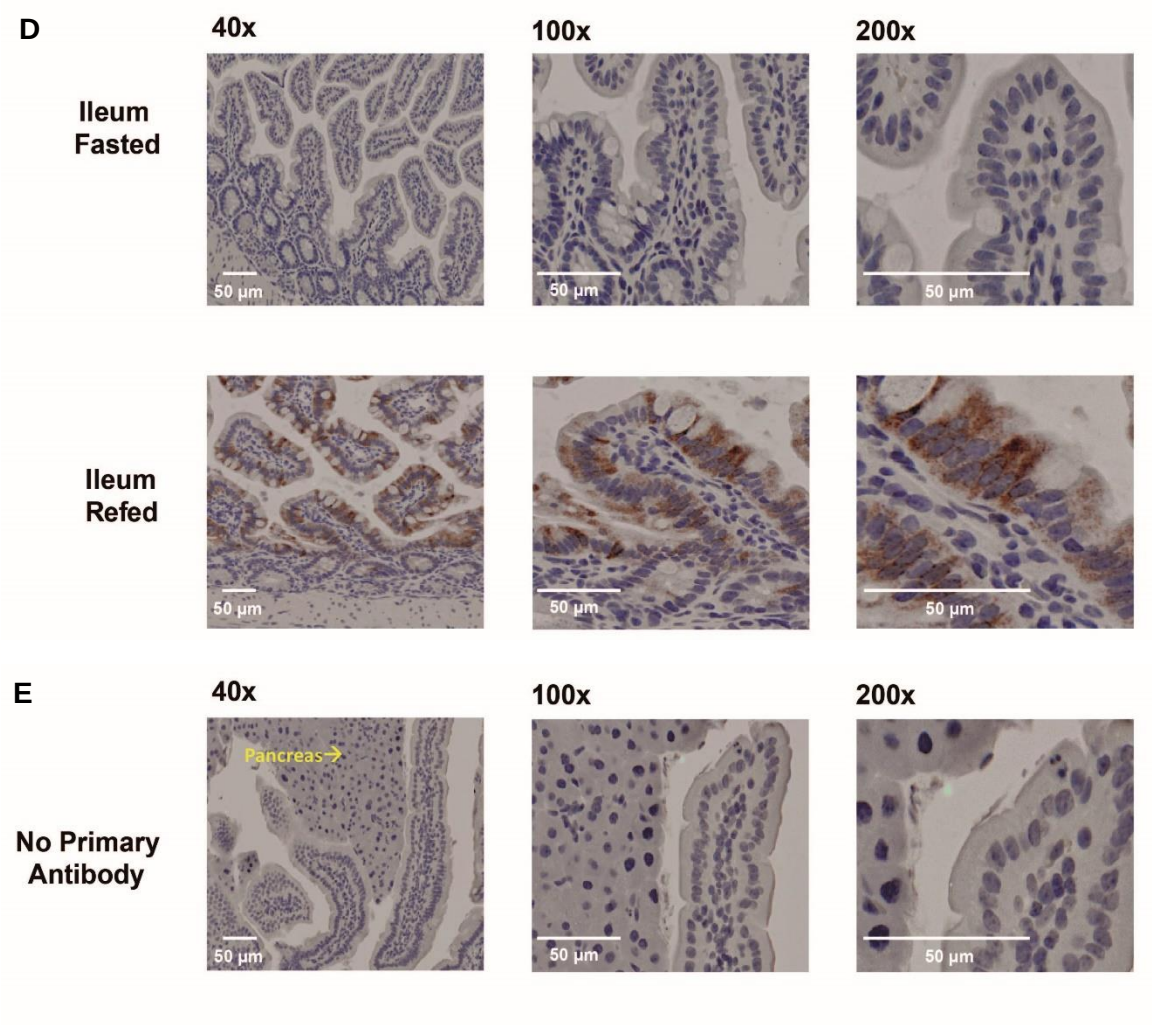


Figure 2.10 Immunohistological analysis of SCD1 shows an increase in staining after refeeding a high carbohydrate low fat diet. Swiss roll sections were prepared for proximal and distal SI for fasted and refed animals (n=2 per group). Increased SCD1 staining in refed tissues of small intestine as seen in gene expression and protein expression (A-D).

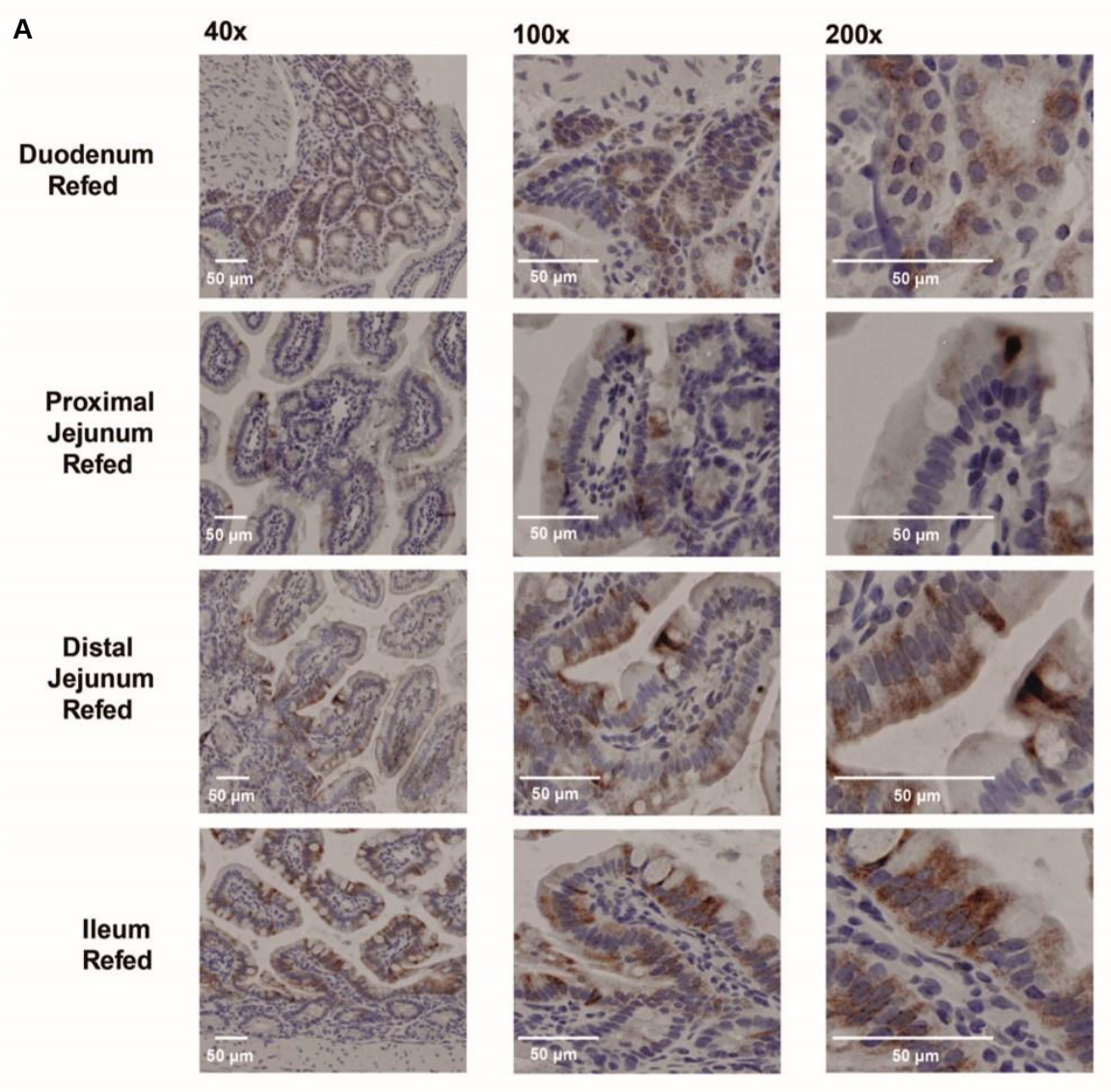


Figure 2.11 Immunohistological analysis of SCD1 shows an increase as well as darker staining in distal SI in comparison to proximal SI with the highest levels of staining in the ileum. Swiss roll sections were prepared for proximal and distal SI for fasted and refed animals (n=2 per group). Increased intensity and amount of SCD1 staining in distal portions of small intestine after refeeding as seen in gene expression and protein expression (A).

Discussion

These data indicate that SCD1 is expressed in the small intestine and is regulated at the transcriptional level by fasting and refeeding a high carbohydrate low-fat diet. Increases in *Scd1* gene expression also translated to increased protein levels. Although the primary site of lipid absorption is the proximal jejunum, SCD1 is expressed at the highest level in the ileum, in comparison to duodenum and jejunum in the refed state (Fig. 2.8B). Similarly, *Scd2* is also expressed in the small intestine and is upregulated in response to high sucrose feeding (Fig. 2.4A-E). Like *Scd1*, *Scd2* also seems to be expressed at the highest level in distal portions of the intestine in comparison to proximal portions in the refed state (Fig. 2.4G). Notably, both *Scd1* and *Scd2* were expressed highest in distal portions of the intestine, namely in the ileum and colon (Fig. 2.3F-G, Fig. 2.4F-G). There was notable induction of *Scd2* expression in the duodenum in response to refeeding, unlike the lack of regulation observed for *Scd1* (Fig. 2.4A). The dual expression of both desaturases in the intestine raise questions about the relative roles of each isoform in the intestine.

Although the primary site of lipid absorption is the jejunum, we find that SCD1 is enriched at the highest level in the ileum (Fig. 2.8B). This raises the question as to what the physiological importance of SCD1 could be in distal portions of the intestine. The higher expression of SCD1 in the distal portions of the intestine raises the possibility that it may be important to cell types that are known to be enriched in the distal regions of the intestine. For instance, enteroendocrine cells like L-cells that secrete the incretin hormone glucagon-like peptide 1 (GLP-1) are enriched in the ileum[46]. GLP-1 is an incretin secreted by L-cells that enhances insulin secretion from the pancreas as well as delays gastric emptying as a means to maintain normoglycemia[46,47]. GLP-1 also reduces food intake and promotes satiety by binding to GLP-1 receptors that are found in the

brain[47,48]. GLP-1 agonists work to prolong the actions of GLP-1 by enhancing insulin secretion and reducing levels of glucagon, a hormone secreted by alpha cells in the pancreas responsible for the release of glucose from the liver[47,49,50]. Interestingly, oleate is a known stimulator of GLP-1 secretion[51]. Given the role of SCD1 in generating oleate, future studies will investigate if SCD1 is expressed in GLP-1 expressing L-cells and whether inhibition of SCD1 has any impact on GLP-1 secretion.

To our knowledge, our discovery of *Scd1* and *Scd2* in the intestine is instance novel discovery of robust and concomitant expression of both isoforms in the same tissue at the same developmental stage. Our studies have established that both these isoforms are regulated by high carbohydrate feeding and could potentially play a significant role in esterification in the intestine. To further investigate what the potential roles of each isoform could be in the intestine as well as whole body lipid homeostasis, this could and should be further explored through intestine-specific SCD1 knockout, intestine-specific SCD2 knockout, as well as intestine-specific double knockout models. A limitation of our study could be that we have not observed the effects of high-fat feeding in a fasting refeeding paradigm. This could be done by exploring effects of palmitate and stearate through tripalmitin and tristearin feeding on *Scd1* regulation, rates of lipid absorption, lipid storage, and lipid secretion in a fasting-refeeding model.

It was recently reported that intestine specific *Scd1* deletion in mice with a point mutation in the Apc gene (*iScd1*$^{-/-}$*Apc*$^{Min/+}$) causes increased tumor burden[52]. However, this observation was reversed with dietary oleate[52]. The potential mechanism behind this reduction in tumor burden was reported to be due to down-regulation of pro-inflammatory genes such as tumor-necrosis factor α (TNFα) and interleukin 1B (IL-1B)[52]. Oleate is a signal transducer that can activate certain nuclear receptors as well as coactivators that are responsible for generating an anti-inflammatory response[52]. In

another study, it has been shown that *Scd1* knockdown in the enterocyte in low density lipoprotein receptor (LDLR) null mice protects mice from weight gain on a Western diet due to decreased synthesis of oleate and consequently decreased incorporation of oleate into lysophosphatidylcholine (LysoPC) and lysophosphatidic acid (LPA)[53]. LysoPC 18:1 and LPA 18:1 have been associated with causing dyslipidemia and systemic inflammation[53]. However, when their synthesis is downregulated with the knockdown of intestinal SCD1, there is decreased dyslipidemia as well as decreased systemic inflammation[53]. Again, this highlights the importance of SCD1 for lipid esterification in the intestine. This suggests a role of intestinal SCD1 in not just maintaining intestine health, but also maintaining overall metabolic health.

Individually speaking, it has been shown that *Scd1-/-* mice exhibit increased energy expenditure and resistance to diet induced obesity[25]. Along with this, tissue specific knockout models also exhibit favorable phenotypes specifically in the liver and skin[28,31]. SCD2 deficiency in mice in the hypothalamus results in increased energy expenditure as well resistance to diet induced weight gain[15]. SCD2 has also been shown to be a potent regulator of adipogenesis as this was observed in 3T3-L1 cells[54]. Downregulation of SCD2 in 3T3-L1 cells was accompanied by a decrease in expression of PPAR gamma mRNA and protein levels[54]. Studies are currently underway to determine the physiological impact of deletion of SCD1 from the intestine. These studies will provide more insight on whether *Scd2* gene expression is upregulated to compensate for loss of SCD1 and address the relative roles of the two desaturase isoforms in the intestine.

Pharmacological regulation of *Scd1* has yet to be completely understood. Thiazolidindiones (TZDs) are a class of drugs that have been shown to enhance insulin sensitivity in diabetics by activating peroxisome proliferator-activated receptor gamma (PPARɣ)[5]. This allows for increased lipid storage and decreased lipotoxicity due to

decreased circulating levels of free fatty acids, in turn promoting normoglycemia in diabetics[5]. In particular, troglitazone, a TZD, has been shown to decrease the expression of *Scd1* in 3T3-L1 adipocytes and liver thereby increasing insulin sensitivity[55,56]. However, this downregulation of *Scd1* in conjunction with improved insulin sensitivity has not always been seen with the use of other TZDs such as rosiglitazone[57]. Thus, how pharmacological therapies enhance insulin sensitivity through a *Scd1*-dependent manner has yet to be further elucidated. In particular, it is important to note that neither depletion nor over expression of *Scd1* has been shown to be completely beneficial. Although global as well as skin-specific deficiency in mice results in resistance to diet induced obesity and increased energy expenditure, this phenotype comes at a cost[31]. Mice also suffer from impaired skin and hair development[31].

It is notable that similar redundant expression has been demonstrated for diacylglycerol acyltransferase (DGAT), the terminal enzyme in triglyceride synthesis. The intestine expresses both DGAT1 and 2 isoforms. Knockout of DGAT1 established that DGAT1 is not essential for triglyceride synthesis within the intestine as well as chylomicron secretion[58]. Here, we see an example in which one isoform compensates for the absence of the other. Additionally, DGAT1 global knockouts exhibit increases in energy expenditure, basal metabolic rate, physical activity, and leptin sensitivity along with hair loss and dry skin: a phenotype that has been observed in SCD1 global as well as skin-specific knockouts[59,60]. This suggests that lipid metabolic enzymes, specifically involved in the synthesis of the storage form of lipids, are essential for the development and function of the skin.

The intestine is the primary site of esterification for the synthesis of triglycerides as well as cholesterol esters. Esterification itself is an important process for lipid absorption and there is evidence that strongly suggests that the synthesis of cholesterol

esters primarily depends on SCD1-derived oleate[13]. Therefore, SCD1 could play an important role in the esterification process in the intestine. With 70% of men and women in the United States being either obese or overweight and evidence suggesting that increased SCD activity is correlated with diseases such as metabolic syndrome, cardiovascular disease, and colon and esophageal cancers[1,5,8,18,19], this suggests that *Scd1* must be tightly regulated through both effective nutritional and pharmacological therapies. In summary, we have discovered two isoforms of stearoyl-CoA desaturase that are not only expressed but also highly regulated by a diet high in sucrose and low in fat in the intestine of the adult mouse. These isoforms show overlapping, as well as some distinctive patterns of regulation along the small intestine in which both isoforms are expressed highest in distal portions of the intestine. These studies provide the premise for future investigations on the role of intestinal SCD1 in regulating intestinal and systemic metabolic health. In particular, future studies will investigate whether *Scd1* is expressed in enteroendocrine cells and whether or not the depletion of SCD1 has any impact on GLP-1 secretion. Future studies will also elucidate the relative contributions of each isoform towards lipid metabolism in the intestine.

Literature Cited

1. Overweight & Obesity Statistics | NIDDK. https://www.niddk.nih.gov/health-information/health-statistics/overweight-obesity. Accessed January 14, 2020.

2. Metabolic Syndrome: Causes, Symptoms, And Treatments | The Grand Healthcare. https://thegrandhealthcare.com/metabolic-syndrome-symptoms-treatments/. Accessed January 15, 2020.

3. Metabolic Syndrome. https://www.medscape.org/viewarticle/484166_2. Accessed January 15, 2020.

4. Metabolic syndrome - Symptoms and causes - Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/metabolic-syndrome/symptoms-causes/syc-20351916. Accessed January 4, 2020.

5. Sampath H, Ntambi & J. Role of stearoyl-CoA desaturase in human metabolic disease. *Future Lipidol*. 2008;3(2):163-173. doi:10.2217/17460875.3.2.163

6. Weng CM, Miyazaki M, Chu K, Ntambi JM. Membrane topology of mouse stearoyl-CoA desaturase. *J Biol Chem*. 2006;281(2):1251-1260. doi:10.1074/jbc.M508733200

7. Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. *Am J Physiol - Endocrinol Metab*. 2009;297(1). doi:10.1152/ajpendo.90897.2008

8. Flowers MT, Ntambi JM. Stearoyl-CoA desaturase and its relation to high-carbohydrate diets and obesity. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2009;1791(2):85-91. doi:10.1016/j.bbalip.2008.12.011

9. ALJohani AM, Syed DN, Ntambi JM. Insights into Stearoyl-CoA Desaturase-1 Regulation of Systemic Metabolism. *Trends Endocrinol Metab*. 2017;28(12):831-842. doi:10.1016/j.tem.2017.10.003

10. Wang J, Yu L, Schmidt RE, et al. Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochem Biophys Res Commun*. 2005;332(3):735-742. doi:10.1016/j.bbrc.2005.05.013

11. Liu X, Strable MS, Ntambi JM. Stearoyl CoA Desaturase 1: Role in Cellular Inflammation and Stress. *Adv Nutr*. 2011;2(1):15-22. doi:10.3945/an.110.000125

12. Rogowski MP, Flowers MT, Stamatikos AD, Ntambi JM, Paton CM. SCD1 activity in muscle increases triglyceride PUFA content, exercise capacity, and PPARδ expression in mice. *J Lipid Res*. 2013;54(10):2636-2646. doi:10.1194/jlr.M035865

13. Dumas SN, Ntambi JM. Increased hydrophilic plasma bile acids are correlated with protection from adiposity in skin-specific stearoyl-coa desaturase-1 deficient mice. *PLoS One*. 2018;13(7). doi:10.1371/journal.pone.0199682

14. Miyazaki M, Dobrzyn A, Elias PM, Ntambi JM. Stearoyl-CoA desaturase-2 gene

expression is required for lipid synthesis during early skin and liver development. *Proc Natl Acad Sci U S A*. 2005;102(35):12501-12506. doi:10.1073/pnas.0503132102

15. De Moura RF, Nascimento LF, Ignacio-Souza LM, et al. Hypothalamic stearoyl-CoA desaturase-2 (SCD2) controls whole-body energy expenditure. *Int J Obes*. 2016;40(3):471-478. doi:10.1038/ijo.2015.188

16. Biochemistry of Lipids, Lipoproteins and Membranes - Google Books. https://books.google.com/books?id=7eKoBAAAQBAJ&pg=PA194&lpg=PA194&dq=where+is+scd2+expressed+in+skin&source=bl&ots=Cku44OXKmj&sig=ACfU3U0quqTP4rMqNL0oEgtMsN9-4yLuUQ&hl=en&ppis=_e&sa=X&ved=2ahUKEwjLsIyO5O3mAhXRslkKHd82BioQ6AEwAnoECAkQAQ#v=onepage&q=where is scd2 expressed in skin&f=false. Accessed January 5, 2020.

17. Miyazaki M, Enrique Gomez F, Ntambi JM. Lack of stearoyl-CoA desaturase-1 function induces a palmitoyl-CoA Δ6 desaturase and represses the stearoyl-CoA desaturase-3 gene in the preputial glands of the mouse. *J Lipid Res*. 2002;43(12):2146-2154. doi:10.1194/jlr.M200271-JLR200

18. Igal RA. Stearoyl CoA desaturase-1: New insights into a central regulator of cancer metabolism. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2016;1861(12):1865-1880. doi:10.1016/j.bbalip.2016.09.009

19. Flowers MT. The Δ9 fatty acid desaturation index as a predictor of metabolic disease. *Clin Chem*. 2009;55(12):2071-2073. doi:10.1373/clinchem.2009.135152

20. Astarita G, Jung KM, Vasilevko V, et al. Elevated stearoyl-CoA desaturase in brains of patients with Alzheimer's disease. *PLoS One*. 2011;6(10):1-9. doi:10.1371/journal.pone.0024777

21. Zheng Y, Eilertsen KJ, Ge L, et al. Scd1 is expressed in sebaceous glands and is disrupted in the asebia mouse [4]. *Nat Genet*. 1999;23(3):268-270. doi:10.1038/15446

22. Sundberg JP, Boggess D, Sundberg BA, et al. Asebia-2J (Scd1(ab2j)): A new allele and a model for scarring alopecia. *Am J Pathol*. 2000;156(6):2067-2075. doi:10.1016/S0002-9440(10)65078-X

23. Cohen P, Miyazaki M, Socci ND, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science (80-)*. 2002;297(5579):240-243. doi:10.1126/science.1071527

24. Sampath H, Ntambi JM. Role of stearoyl-CoA desaturase-1 in skin integrity and whole body energy balance. *J Biol Chem*. 2014;289(5):2482-2488. doi:10.1074/jbc.R113.516716

25. Ntambi JM, Miyazaki M, Stoehr JP, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A*. 2002;99(17):11482-11486. doi:10.1073/pnas.132384699

26. Miyazaki M, Kim YC, Ntambi JM. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. *J Lipid Res*. 2001;42(7):1018-1024.

27. Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, Ntambi JM. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J Biol Chem*. 2000;275(39):30132-30138. doi:10.1074/jbc.M005488200

28. Miyazaki M, Flowers MT, Sampath H, et al. Hepatic Stearoyl-CoA Desaturase-1 Deficiency Protects Mice from Carbohydrate-Induced Adiposity and Hepatic Steatosis. *Cell Metab*. 2007;6(6):484-496. doi:10.1016/j.cmet.2007.10.014

29. Hyun CK, Kim E Do, Flowers MT, et al. Adipose-specific deletion of stearoyl-CoA desaturase 1 up-regulates the glucose transporter GLUT1 in adipose tissue. *Biochem Biophys Res Commun*. 2010;399(4):480-486. doi:10.1016/j.bbrc.2010.07.072

30. Liu X, Miyazaki M, Flowers MT, et al. Loss of Stearoyl-CoA Desaturase-1 Attenuates Adipocyte Inflammation Effects of Adipocyte-Derived Oleate. 2009. doi:10.1161/ATVBAHA.109.195636

31. Sampath H, Flowers MT, Liu X, et al. Skin-specific deletion of stearoyl-CoA desaturase-1 alters skin lipid composition and protects mice from high fat diet-induced obesity. *J Biol Chem*. 2009;284(30):19961-19973. doi:10.1074/jbc.M109.014225

32. Iqbal J, Hussain MM. Intestinal lipid absorption. *Am J Physiol - Endocrinol Metab*. 2009;296(6). doi:10.1152/ajpendo.90899.2008

33. Stahl A, Hirsch DJ, Gimeno RE, et al. Identification of the major intestinal fatty acid transport protein. *Mol Cell*. 1999;4(3):299-308. doi:10.1016/S1097-2765(00)80332-9

34. Poreba MA, Dong CX, Li SK, Stahl A, Miner JH, Brubaker PL. Role of fatty acid transport protein 4 in oleic acid-induced glucagon-like peptide-1 secretion from murine intestinal L cells. *Am J Physiol - Endocrinol Metab*. 2012;303(7). doi:10.1152/ajpendo.00116.2012

35. Yen CLE, Nelson DW, Yen MI. Intestinal triacylglycerol synthesis in fat absorption and systemic energy metabolism. *J Lipid Res*. 2015;56(3):489-501. doi:10.1194/jlr.R052902

36. Ho SY, Storch J. Common mechanisms of monoacylglycerol and fatty acid uptake by human intestinal Caco-2 cells. *Am J Physiol - Cell Physiol*. 2001;281(4 50-4). doi:10.1152/ajpcell.2001.281.4.c1106

37. Murota K, Matsui N, Kawada T, Takahashi N, Fushuki T. Inhibitory effect of monoacylglycerol on fatty acid uptake into rat intestinal epithelial cells. *Biosci Biotechnol Biochem*. 2001;65(6):1441-1443. doi:10.1271/bbb.65.1441

38. Murota K, Storch J. Uptake of Micellar Long-Chain Fatty Acid and sn-2-Monoacylglycerol into Human Intestinal Caco-2 Cells Exhibits Characteristics of Protein-Mediated Transport. *J Nutr*. 2005;135(7):1626-1630. doi:10.1093/jn/135.7.1626

39. Zhang J, Kelley KL, Marshall SM, et al. Tissue-specific knockouts of ACAT2 reveal that intestinal depletion is sufficient to prevent diet-induced cholesterol accumulation in the liver and blood. *J Lipid Res*. 2012;53(6):1144-1152. doi:10.1194/jlr.M024356

40. Kim HJ, Miyazaki M, Ntambi JM. Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism. *J Lipid Res*. 2002;43(10):1750-1757. doi:10.1194/jlr.M100433-JLR200

41. Ntambi JM. Dietary regulation of stearoyl-CoA desaturase 1 gene expression in mouse liver. *J Biol Chem*. 1992;267(15):10925-10930.

42. He F. Bradford Protein Assay. *BIO-PROTOCOL*. 2011;1(6). doi:10.21769/bioprotoc.45

43. Chao A. Non-parametric estimation of the classes in a population. *Scand J Stat*. 1984;11(4):265-270. doi:10.2307/4615964

44. Chao A, Chazdon RL, Colwell RK, Shen T-J. A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecol Lett*. 2004;8(2):148-159. doi:10.1111/j.1461-0248.2004.00707.x

45. Kaestner KH, Ntambi JM, Kelly TJ, Lane MD. Differentiation-induced gene expression in 3T3-L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. *J Biol Chem*. 1989;264(25):14755-14761.

46. Reimann F, Gribble FM. Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes*. 2002;51(9):2757-2763. doi:10.2337/diabetes.51.9.2757

47. Meier | December ; J. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2012;8:728-742. doi:10.1038/nrendo.2012.140

48. Meier JJ, Schmidt WE, Nauck MA. *Glucagon-like Peptide 1 as a Regulator of Food Intake and Body Weight: Therapeutic Perspectives*. www.elsevier.com/locate/ejphar. Accessed March 27, 2020.

49. Liu Z, Kim W, Chen Z, et al. Insulin and glucagon regulate pancreatic α-cell proliferation. PLoS ONE. doi:10.1371/journal.pone.0016096

50. Zhang H, Qiao A, Yang L, et al. Structure of the glucagon receptor in complex with a glucagon analogue. *Nature*. 2018;553(7686):106-110. doi:10.1038/nature25153

51. Clara R, Langhans W, Mansouri A. Oleic acid stimulates glucagon-like peptide-1 release from enteroendocrine cells by modulating cell respiration and glycolysis.

Metabolism. 2016;65(3):8-17. doi:10.1016/j.metabol.2015.10.003

52. Ducheix S, Peres C, Härdfeldt J, et al. Deletion of Stearoyl-CoA Desaturase-1 From the Intestinal Epithelium Promotes Inflammation and Tumorigenesis, Reversed by Dietary Oleate. *Gastroenterology*. 2018;155(5):1524-1538.e9. doi:10.1053/j.gastro.2018.07.032

53. Mukherjee P, Hough G, Chattopadhyay A, et al. Role of enterocyte stearoyl-Co-A desaturase-1 in LDLR-null mice. *J Lipid Res*. 2018;59(10):1818-1840. doi:10.1194/jlr.M083527

54. Christianson JL, Nicoloro S, Straubhaar J, Czech MP. Stearoyl-CoA desaturase 2 is required for peroxisome proliferator-activated receptor γ expression and adipogenesis in cultured 3T3-L1 cells. *J Biol Chem*. 2008;283(5):2906-2916. doi:10.1074/jbc.M705656200

55. Kakuma T, Lee Y, Unger RH. *Effects of Leptin, Troglitazone, and Dietary Fat on Stearoyl CoA Desaturase*. www.academicpress.com. Accessed January 14, 2020.

56. Kurebayashi S, Hirose T, Miyashita Y, Kasayama S, Kishimoto T. Thiazolidinediones downregulate stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. *Diabetes*. 1997;46(12):2115-2118. doi:10.2337/diab.46.12.2115

57. Singh Ahuja H, Liu S, Crombie DL, et al. *Differential Effects of Rexinoids and Thiazolidinediones on Metabolic Gene Expression in Diabetic Rodents*.; 2001.

58. Buhman KK, Smith SJ, Stone SJ, et al. DGAT1 is not essential for intestinal triacylglycerol absorption or chylomicron synthesis. *J Biol Chem*. 2002;277(28):25474-25479. doi:10.1074/jbc.M202013200

59. Smith SJ, Cases S, Jensen DR, et al. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat Genet*. 2000;25(1):87-90. doi:10.1038/75651

60. Chen HC, Smith SJ, Tow B, Elias PM, Farese R V. Leptin modulates the effects of acyl CoA:diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands. *J Clin Invest*. 2002;109(2):175-181. doi:10.1172/JCI13880

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Supplemental Materials

High Sucrose Very Low Fat (HSVLF) Diet

Formula	Weight (g/Kg)
Casein, "Vitamin-Free" Test	200
DL-Methionine	3
Sucrose	486.6
Corn Starch	41.21
Maltodextrin	150
Corn Oil	10
Cellulose	50
Mineral Mix, AIN-93G-MX (94046)	35
Potassium Phosphate, monobasic	8.79
Calcium Carbonate	3.75
Magnesium Oxide	1.35
Ferric Citrate	0.29
Vitamin Mix, Teklad (40060)	10
TBHQ, antioxidant	0.01

Table S1: High Sucrose Very Low Fat (HSVLF) Diet Composition. The HSVLF diet is 49% sucrose and 1% corn oil by weight. Approximately 76.7% of the calories from this diet are from carbohydrate, 20.7% from protein, and 2.5% from fat.