EVALUATION OF MOLECULAR MECHANISMS IMPACTING BEEF QUALITY AND CARCASS CHARACTERISTICS

by

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ABSTRACT

Tenderness has been stated as being one of the most important traits for consumers with regards to purchasing steak. However, too often there is much variation in tenderness in steaks that grade the same quality. Research in the past has done little to expand on the impact that genetics has on meat tenderness. This study was designed to attempt to illuminate this question by looking at the genes being differentially expressed between quality grades, and the pathways they impact. Angus crossbred steers (15) were fed to the end points of Standard, Select and Choice. Intermuscular and subcutaneous adipose tissue and muscle samples were snap frozen for gene expression analysis, as well as a loin kept from each animal for shear force analysis. Shear force analysis showed a significant difference between Choice and Standard graded animals ($P<0.05$), showing that the Choice animals in this study were more tender than Standard. RNA extracted from the adipose and muscle samples were sent to Novogene for RNAseq analysis. The results of the gene expression analysis showed 4 genes down regulated and 29 up regulated in the comparison of Select to Standard adipose tissue, with 8 genes down regulated and 15 up regulated with Select to Choice adipose tissue. The largest difference occurred between Standard to Choice adipose tissue with 49 genes down regulated and 113 up regulated. With regards to the muscle, 15 genes were down regulated while 20 were up regulated in the Standard to Choice. In the Select to Choice, 1 gene was down regulated with 4 up regulated. When comparing Select to Standard, there was not a large enough difference in genes being expressed. This study emphasized the need for additional functional studies on the impacts of gene expression on marbling deposition.

INTRODUCTION

The livestock industry is one of the largest industries in the world. Agriculture is said to occupy roughly 30% of the planets livable surface area (Thornton, 2010), while employing at least 1.3 billion people globally (Thornton et al., 2006). The world population is estimated to be 9.15 billion in the year 2050 (UNPD, 2008) and as a person's income grows so does the spending on livestock products (Steinfeld et al., 2006), specifically with regards to meat products. Tenderness has been stated as the most important factor affecting palatability, or a consumer's perception of palatability, of meat (Dikeman, 1987; Savell et al., 1987; Morgan et al., 1991; Koohmaraie, 1994; Miller et al., 1995; Huffman et al., 1996). The issue however, is that it is unknown who all is satisfied with their meat product, as only 0.1% of those who are unsatisfied with their steak return the product or complain (Wilkes, 1992). Not surprisingly, Morgan (1992) reported an instance when a supermarket chain asked customers to return all meat they deemed unsatisfactory. Over a three year period they received $364,000 worth of meat, with 78% of returned product being due to tenderness issues.

Platter et al. (2003) found that when consumers evaluated strip loin steaks from different USDA Quality grade categories, variation in tenderness was observed within a category. This suggests that quality grade alone will not guarantee tenderness. Improved tenderness and reduction in tenderness variation are very important to maintaining or increasing demand for beef. Consumers have stated that they are willing to spend more money on meat that is known to be more tender (Boleman et al., 1995; Acebron and Dopico, 2000; Miller et al., 2001; Shackelford et al., 2001).

One of the main issues with meat and tenderness is that the tenderness of a particular cut of meat will not be known until it is being consumed (Koohmaraie et al., 1994). In addition, an increase in marbling has been shown in some studies to decrease shear force (Park et al., 2000; Kim and Lee, 2003; Li et al., 2006), while others have shown marbling to have no impact on tenderness and the consumer's perception of it (Brooks et al., 2000). Improvements have been made with regards to tenderness in beef (Guelker et al., 2013), but there is still progress to be made in producing consistent, uniformly tender meat products, as well as determining all factors that impact tenderness.

One area that can be used to improve overall meat quality is genetics. While much research has been completed to determine specific genes that code for tender meat, a single gene has yet to be found that explains more than 50% of the observed variation in the tenderness of aged beef (Koohmaraie et al., 1995). Due to this, a method needs to be determined that will give producers a way to select for cattle that will produce consistently more tender meat.

While advances have been made with regards to genetics and the influence they have with regards to meat tenderness, the question of which and how specific genes determine meat quality remains. Previous work at MSU (Engle et al., 2015) utilized a cohort of Hereford steers from Ft. Keogh Agricultural Research station that produced carcasses that graded Standard, Select and Choice. *Longissimus lumborum* muscle was sampled at slaughter and used for gene expression analysis. Upon analysis of differentially expressed genes, a significant number of differences were observed between Choice and Standard carcass pools (1258 genes <0.01). A functional analysis

was run using DAVID bioinformatics software, which revealed differences in the underlying pathways regulating muscle cell growth and proliferation. Biological processes such as growth, muscle hypertrophy, protein kinase activity, and lipid biosynthetic pathway were found to be enriched in the differentially expressed gene set. The purpose of this study was to expand upon and confirm previous results. The objective of this work was to provide new insight into the molecular and genetic basis of meat quality grade.

LITERATURE REVIEW

Muscle Structure

The muscle is made up of many parts, primarily myofilaments, which contain thousands of myofibrils (Greaser, 1991). These myofibrils are made up of repeating sarcomeres (Bailey, 1972), which is considered the basic contractile unit of myofibrils (Clark et al., 2002); the myofibril is made up of many different parts: the Z-line, I-band, A-band, M-line, titin, nebulin, myosin, actin, alpha actinin, and the thin and thick filaments.

Z-line

The Z-line is the lateral edge of the sarcomere, and is also where titin, nebulin, and the thin filaments are attached (Clark et al., 2002). Due to the Z-line being an "anchor", they are the primary channels of force generated by a contraction.

Thin and Thick Filament

Thin filaments are made up of an actin double helix (Marston and Smith, 1985), with nebulin, tropomyosin and the troponin complex along its length and capped at the end by tropomyosin and CapZ. They are attached in the Z-line, extends past the I-band and on towards the middle of the sarcomere. Once it reaches the A-band, the thin filaments interdigitate with the thick filaments (Clark et al., 2002). Troponin, which is connected to tropomyosin, blocks the myosin head binding site on the actin. When it is activated by calcium, tropomyosin moves, allowing myosin to bind to actin. In order for the myosin head to release actin, ATP needs to bind to the myosin head, causing the

reduction in the binding constant between myosin and actin and thus its release. The ATP is then hydrolyzed to ADP and inorganic phosphate (Geeves and Holmes, 1999).

Titin

While titin is the largest protein currently known in mammals and is the third most abundant (Lonergan et al., 2010), it was one of the last to be discovered. It wasn't until Wang et al. (1979) and Maruyama et al. (1977) looked at the insoluble residue that was often discarded. It was then discovered, along with nebulin, and has a major role in the muscle. Titin extends from the Z-line to the M-band, and is highly modular, with 90% of its mass consisting of repeating immunoglobulins and fibronectin-III domains (Kontrogianni-Konstantopoulos et al., 2009). The ends of titin have opposing polarity, overlapping in both the Z-lines and M-bands, forming a continuous system (Kontrogianni-Konstantopoulos et al., 2009). Titin has many structural properties that are responsible for making it so important in muscle- 1) titin in the I-band region have elastic properties, allowing the "spring" to move the sarcomere to its resting size; 2) the repeating nature of titin, as well as its early assembly during myofibrillogenesis and the way it interacts with other components make it a candidate as a sarcomeric template and stabilizer; 3) the C-terminal region on titin has a Ser/Thr kinase domain, suggesting that titin may play a part in signaling pathways (Clark et al., 2002).

Nebulin

Another giant protein, nebulin was discovered about the same time by Wang et al. (1979). It binds along the length of the thin filaments (Kontrogianni-Konstantopoulos et al., 2009), and is made up of a C-terminal that is moderately bound to the Z-lines, as well

as an N-terminal that extends towards the capped end of the thin filament (Clark et al., 2002). It comes in contact with the capping proteins on the thin filaments, as well as having its C-terminal bind to titin. Due to nebulin's rigid structure, it has been suggested as a candidate as a molecular ruler for determining the length of the thin filaments (Clark et al., 2002). Titin is also thought to help bind nebulin to the z-line (Kontrogianni-Konstantopoulos et al., 2009).

Desmin

Desmin is a part of the intermediate filaments, and is thought to surround the Z-line and connect adjacent myofibrils at the level of the Z-line (Huff-Lonergan and Lonergan, 1999). It also is thought to connect the peripheral myofibrils to the sarcolemma (Richardson et al., 1981). Desmin is known to be degraded early postmortem (Hwan and Bandman, 1989; Huff-Lonergan et al., 1996; Huff-Lonergan and Lonergan, 1999), with studies showing a quicker degradation of desmin in samples with low shear force and a higher water holding capacity, than in samples with a higher shear force and lower water holding capacity (Huff-Lonergan et al., 1996; Huff-Lonergan and Lonergan, 1999; Zhang et al, 2006). Researchers have reported that at least half of desmin has undergone proteolysis by 72 hours postmortem (Taylor et al., 1995), with others reporting up to 94% of desmin being degraded by 15 days postmortem (Veiseth et al., 2004). Due to desmin connecting adjacent myofibrils together as well as connecting myofibrils to the sarcolemma, the degradation of desmin could compromise the structure of the muscle fiber (Robson, 1995; Huff-Lonergan and Lonergan, 1999).

Troponin-T

Troponin-T is the subunit of Troponin that connects the complex to tropomyosin, one of the many thin filament components which has been linked to regulation of striated muscle contraction (Pearlstone and Smillie, 1982; Huff-Lonergan et al., 1996). It has been shown to be prone to postmortem degradation (Penny and Dransfield, 1979; Huff-Lonergan et al.,1996). Troponin-T has been shown to degrade more rapidly in beef that is more tender than in beef that is less tender (Koohmaraie et al., 1984; Ho et al., 1994; Huff-Lonergan et al.,1996).

Filamin

Filamin is thought to be located around the outer portion of the Z-line (Price et al., 1994). During in vivo conditions, it exists as a dimer with two identical 250 kDa subunits, which attach at the C terminal, forming a V-shaped molecule. The V-shape structure of filamin makes it ideal to cross-link actin filaments (Huff-Lonergan et al., 1999). During postmortem aging, calpains have been shown to break down filamin resulting in the 250 kDa subunit being broken into a 240 kDa and 10 kDa subunits (Davies et al., 1978). This cleavage occurs near the C-terminal domain, this breakdown hinders the ability of filamin to cross-link actin filaments, but not its ability to bind to actin (Huff-Lonergan et al., 1999). It has been shown to be degraded postmortem, as early as three days in tender samples, and as late as fourteen days postmortem in less tender samples (Huff-Lonergan et al., 1996). It also appears to degrade slower than titin and nebulin (Huff-Lonergan and Lonergan, 1999).

Conversion of Muscle to Meat

The time between death and rigor mortis varies between animals, and is determined by two considerations- 1) the pH of the muscle at the moment of death (which is determined by activity immediately preceding death), and 2) the glycogen reserve of the muscle (Bate-Smith and Bendall, 1947). After exsanguination, metabolism switches from aerobic to anaerobic due to a lack of oxygen, causing lactic acid to be produced. The lack of blood flow to carry lactic acid away results in a drop in muscle pH (Savell et al., 2005). During this period, few rigor bonds form, resulting in the muscle being extensible due to glycogen being readily available to produce ATP (Przybylski and Hopkins, 2015). As long as there is sufficient glycogen present for the synthesis of ATP the muscle does not pass into rigor (Whitaker, 1960). A study by Bodwell et al. (1965) showed glycogen decreased up to 54% within the first 12 hours post mortem, to less than 3% 48 hours post mortem. Furthermore, lactic acid increased almost 3.5 times in the initial 6 hours post mortem. Creatine phosphate is a rapid method of restoring ATP by phosphorylating ADP to ATP. The presence of ATP in the muscle causes the myosin head to release actin and prevents permanent cross-bridge formation. After creatine phosphate has been depleted, the levels of ATP decline rapidly, resulting in the loss of ability of myosin to separate from actin. This causes permanent bonds to form between myosin and actin, leading to the shortening of the sarcomere.

Tenderness

As was stated previously, tenderness has been rated as the most important characteristic of meat consumers eat (Dikeman, 1987; Savell et al., 1987; Morgan et al., 1991; Koohmaraie, 1994; Miller et al., 1995; Huffman et al., 1996). With that being said, there are many factors that can influence the tenderness of meat. Of those factors, the ones that tend to have the biggest influence are temperature, pH levels, and the amount of stress at the time of slaughter.

Temperature

It has long been established that temperature can play an important part in the tenderization of meat (Locker and Hagyard, 1963; Moeller et al., 1976; Parrish et al., 1973; Pierson and Fox, 1976). Temperature can have 3 main outcomes of the meat: cold shortening, thaw rigor, and heat rigor. Cold shortening occurs when a carcass goes through a rapid decrease in temperature prior to the onset of rigor, reaching temperatures below and above 0°C. When this occurs, the sarcoplasmic reticulum is incapable to function correctly and is unable to bind calcium as it should, resulting in an over-abundance of calcium in the sarcoplasm (Savell et al., 2005). Because ATP is still available, the muscle continues to contract resulting in shortening of rigor beyond what would normally occur, for all intents and purposes eliminating the I-band (Locker and Daines, 1976). This can result in the sarcomeres being shortened up to 35% of their normal length (Locker and Hagyard, 1963). This phenomenon is referred to as cold shortening and results in the meat being tough.

When a carcass is frozen pre-rigor, it too has an impact on the quality of the meat. This is referred to as thaw rigor. When the frozen muscle thaws, the fibers in the muscle contract due to a sudden release of calcium into the sarcoplasm. The outcome is the muscle fibers shortening 60-80% of its original length, as well as resulting in the muscle being tough (Aberle, 2001). The last way temperature can influence tenderness is heat rigor. This is arises when a muscle is maintained at high temperatures, above 35°C and as high as 50°C. The high temperatures result in a rapid depletion of ATP, causing the muscle fibers to shorten and for rigor to set in early (Warner et al., 2014). When heat rigor occurs, the sarcomeres shorten up to 30% of their normal length (Lonergan et al., 2010).

pH

It has been shown that, similar to temperature, early post-mortem pH plays an important role in the rate of meat tenderization (OHalloran, Troy, & Buckley, 1997). The overall rate of pH fall is determined by the rate of ATP-turnover (Bendall, 1978; OHalloran et al., 1997). Due to this, there is much variation in pH decline due to the amount of free calcium available at the time of slaughter.

In a normal animal that has not been abnormally stressed, the pH in the muscle decreases from 7.0 upon slaughter to approximately 5.3-5.8 (Smulders et al., 1992). However, Silva et al. (1999) found that meat with a normal pH was significantly ($p<0.05$) tougher than moderate dark, firm and dry meat (5.8-6.2), as well as dark, firm and dry (DFD) meat (6.2-6.7). Meat that is often referred to as dark, firm and dry tends to be darker in color, is more susceptible to bacterial spoilage, and has reduced flavor. This

occurs due to the glycogen stores being depleted prior to harvest, which will result in less lactic acid building up post-mortem, making it not possible for the pH to drop as it should (Lonergan et al., 2010).

Stress

Whenever an animal perceives something as not normal or unsafe, that animal is stressed. Stott (1981) defined stress as an environmental condition that is detrimental to the well-being of an animals. When stress occurs, it shifts the body away from homeostasis and the degree of the perceived threat will determine how far away from the steady state the body will go (Lee, 1965). When being taken from feedlot to the plant, animals undergo many different stresses: deprivation of food and water, noises, unfamiliar odors, vibration and changes to acceleration, extremes of temperature and humidity, close confinement, overcrowding and the biggest one, the breakdown of social groupings (P. D. Warriss, 1990; Immonen et al., 2000). When stress occurs, epinephrine is released, which acts to accelerate glycogen metabolism (Ashmore et al., 1973). This results in there being less glycogen available for the body to break down post-mortem, causing the carcass to have a higher ultimate pH than non-stressed animals (Grandin, 1980). The end result is an animal that is referred to as a dark cutter, which results in a carcass that tends to have a reduced shelf life (Grandin, 1980; Mcveigh et al., 1980; Lacourt and Tarrant, 1985), increased spoilage (Gill & Newton, 1981; Nicol, Shaw, & Ledward, 1970; P. D. Warriss, 1990), altered color (Lawrie, 1958; Bartos et al., 1993; Kreikemeier et al., 1998; Mounier et al., 2006), higher ultimate pH (Ashmore et al.,

1973), increased water-holding capacity (Apple et al., 2005; Zhang et al., 2005), and is less palatable (Dransfield, 1981; Viljoen et al., 2002; Wulf et al., 2002).

Calpain and Calpastatin

A key element in the transitioning of muscle to tender meat is the weakening of myofibers (Ouali et al., 2006). When considering the system responsible for the tenderization of meat, three characteristics must be met before the system is considered a candidate: 1.) be located within the skeletal muscle; 2.) have access to the substrate; 3.) have the ability to degrade the same proteins that are degraded during post mortem storage. The proteolytic systems that have the possibility of being involved in post-mortem degradation are: 1.) the lysosomal, cathepsins; 2.) the multicatalytic proteinase proteosomes; and 3.) the calpains (Koohmaraie, 1994). Out of the three suggested, the system generally accepted as the cause of this weakening is the calpain proteolytic system (Veiseth and Koohmaraie, 2005). The calpain system is made up of three main parts: µ-calpain, m-calpain, and calpastatin which is the endogenous inhibitor of the calpains (Croall and Demartino, 1991; Shackelford et al., 1994). Whipple et al. (1990) measured calcium-dependent protease (CDP)-I, -II, and CDP inhibitor (INH), as well as Warner-Bratzler shear force and other tenderness-related factors in *Bos taurus* and *Bos indicus* crossbred cattle. They reported that, out of the factors they measured, INH activity was the only trait to have a significant residual correlation with the 14 day WBS and myofibril fragmentation indices. There have also been studies that indicate the CDP mechanism being activated by infusing $CaCl^2$ improved tenderness (Koohmaraie et al.,

1989; Koohmaraie et al., 1990), while inhibiting CDP by infusing $ZnCl^2$ caused no change (Koohmaraie, 1990). Together, these studies give support to the belief of the CDP system's involvement in postmortem tenderization (Whipple et al., 1990). However, Shackelford et al. (1991) performed a similar study and the results were contrary to that of Whipple et al. (1990), showing none of the traits correlating significantly with the 14 day Warner-Bratzler shear force. Though there is evidence and most accept that the calpain proteolytic system is responsible for improvement in meat tenderness, the exact process is unknown.

Adipose Tissue

Adipocytes, which are also known as fat cells, are one of many ways energy is stored in the body. When an animal reaches market weight, roughly 80-90% of total body energy is stored as adipose tissue (Walton and Etherton, 1986), while the adipose tissue itself is made up of 70-90% fat, 5-20% water and roughly 5% connective tissue (Nurnberg et al., 1998). Adipocytes originate from mesenchymal stem cells (Scanes, 2003; Du et al., 2010) that reside in the vascular stroma of adipose tissue, as well as in the bone marrow (Tang and Lane, 2012). The exact pathway that causes adipocytes to differentiate from mesenchymal cells to preadipocytes is not fully understood. However, it is believed that transcription factors Wnt and hedgehog ligands play a part.

When preadipocytes arrive at the growth arrest step of the cell cycle, differentiation to adipocytes begins with a "cocktail" of inducers, including a high level of insulin (Macdougald and Lane, 1995) and dexamethasone. These inducers activate the

IGF1-, glucocorticoid- and cAMP-signaling pathways. After a delay of ~16 to 20 hours after induction, preadipocytes reenter the cell cycle (Student et al., 1980; Davis and Zur Nieden, 2008) and go through many rounds of mitosis, after which the cells exit the cycle, lose their fibroblastic morphology, accrue cytoplasmic triglyceride and acquire the appearance and metabolic features of adipocytes (Green and Kehinde, 1974; Student et al., 1980).

In cattle, the fat cell diameter of subcutaneous fat tissue grows rapidly up to 12 months. After this growth is continued but at a reduced rate up to 2 years of age (Wegner et al., 1998). Cianzio et al. (1985) saw that, as age increased in crossbred steers fed the same growing-finishing diet, the number of adipocytes/gram of adipose tissue decreased up to 15 months of age in six different adipose depots. From 15 to 19 months, the rate of decrease slowed or remained constant. However, the diameter of adipocytes increased up to 15 months of age in the steers, with a slower increase from 15 to 17 months of age.

Adipose tissue has been recognized as a major regulator of systemic metabolism, extending beyond energy buffering (Ouchi et al., 2011). This is done by secreting specialized proteins that exert autocrine, paracrine and endocrine functions. These proteins, known as adipokines, are produced by cellular components of adipose tissue, such as adipocytes and cells of the stromal vascular fraction, including immune, vascular and adipocyte progenitor cells (Jacobi et al., 2006; Contreras et al., 2017). There are currently over 300 secretory products, though the list is constantly expanding (Lehr et al., 2012). Two of the main proteins are adiponectin and leptin which are produced almost exclusively by adipose tissue; while others, such as resistin and retinol binding protein 4,

are produced by adipose tissue and the liver. Similarly, other secretory products, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) are produced by adipocytes, as well as resident immune cells in adipose tissue (Contreras et al., 2017).

Adiponectin is known as ACRP30, and is primarily secreted by adipocytes, but is also produced by cardiomyocytes and skeletal muscle (Jortay et al., 2012). It is known to improves insulin sensitivity and promote lipogenesis in adipocytes, and fatty acid β-oxidation in myocytes and hepatocytes (Stern et al., 2016).

Leptin, which was one of the first adipokines characterized in mammals, is expressed not only by adipose tissue, but also by myocytes, mammary gland and gastric mucosa epithelial cells (La Cava and Matarese, 2004), and is known to decrease lipogenesis while increasing lipolysis and fatty acid oxidation (William et al., 2002). Regulated by genetic, nutritional and environmental factors (Harper and Pethick, 2004), adipose tissue development and deposition can be influenced by breed, sex and diet.

Breed

Genetic-based differences in intramuscular fatty acid composition of longissimus muscle have been noted by many researchers (Robelin, 1986; Enser, 1991; Ender et al., 1997). A study by Huerta-Leidenz et al. (1993) reported differences in fatty acid composition of subcutaneous adipose tissue between Hereford and Brahman cows. Average fat thickness was 1.7 and 1.3 cm, respectively. Herefords had a higher amount of saturated fatty acids and a lower percentage of mono- and polyunsaturated fatty acids in subcutaneous adipose tissue when compared to Brahman. Zembayashi and Nishimura

(1996) proposed, based off of their studies with different Japanese Black breeds, that leaner steers had more saturated fatty acids in subcutaneous and intramuscular fat.

Sex

When animals have been harvested at equal slaughter weights, intact males are typically leaner than females. A study done in pigs showed that intact males tend to be leaner than gilts which in turn are leaner than male castrates (Enser, 1991), while the relative concentration of polyunsaturated fatty acids and linoleic acid decreases in the order of intact males > females > male castrates, whereas the saturated fatty acid percentage increases (Nurnberg and Ender, 1989). These differences in fatty acids have been seen in cattle (Enser, 1991; Malau-Aduli et al., 1998), pigs (Cameron and Enser, 1991), and lambs (Nurnberg et al., 1996) and are caused by the negative relationship between concentrations of fate and polyunsaturated fatty acids in the carcass.

Diet

The level and the chemical composition of feed can affect fat deposition in animals (Nurnberg et al., 1998). A study by Wegner and Matthes (1994) showed that when Herefords and Black Pied steers were managed under extensive conditions to achieve almost linear growth from 140 to 500 days of age, carcasses from Herefords had lower muscle fibers diameters and smaller adipocytes than Black Pied animals.

Lipogenesis

Lipogenesis refers to the synthesis of fat. In cattle, this mainly occurs in adipose tissue, though some does also occur in the liver. It occurs due to the synthesis of fatty

acids which leads to the synthesis of triglycerides. This process occurs differently in ruminants than it does in other species. It is short chain fatty acids that are the source of energy for ruminants, rather than glucose which is commonly the energy source in other mammals. Lipogenesis can also refer to both the hypertrophy and hyperplasia of adipose tissue.

Fatty acid synthesis takes places in the cytoplasm with the help of ATP, NADPH, biotin, bicarbonate, and Mn++. The main sites for this pathway are the liver, kidneys, brain, lungs, mammary glands, and adipose tissue. Acetyl-CoA is the two-carbon building block molecule which is added to malonyl-CoA, formed via carboxylation of acetyl-CoA. The enzyme catalyzing this reaction is acetyl-CoA carboxylase.

The first step is transfer of acetyl-CoA to the cysteine-SH group by acetyl transacylase and malonyl-CoA on 4'-phosphopantetheine of ACP by malonyl transacylase. These two sites belong to different units of the dimer enzyme complex called fatty acid synthase.

In the second step, the acetyl group joins the malonyl residue by 3-ketoacyl synthase and CO_2 is released, yielding 3-ketoacyl. 3-Ketoacyl is reduced in third step utilizing NADPH and 3-ketoacyl reductase, forming 3-hydroxyacyl. The fourth step is dehydration catalyzed by hydratase. This step creates a double bond between C2 and C3. This bond is reduced by enoyl reductase enzyme. The second reduction also needs NADPH. Finally acyl-S enzyme is formed. This acyl residue is then shifted to the empty cysteine-SH group and a new malonyl residue arrives at –SH of 4'-phosphopantetheine.

The reactions are repeated until the desired length is achieved. Thioesterase releases the completed fatty acid by hydration.

Three main factors affect lipogenesis: nutrition, hormones, and genetics.

Nutritional. Lipogenesis is extremely responsive to the diet of an animal and the changes that occur in the diet. A diet that has a large amount of carbohydrates stimulates lipogenesis in both the liver as well as adipose tissue. This results in high levels of triglycerides in the plasma postprandial (Kersten, 2001). However, a diet that contains many polyunsaturated fatty acids causes a decrease in lipogenesis by suppressing gene expression in the liver, including fatty acid synthase, stearoyl-CoA desaturase, and S14, a protein that is known to be under thyroid hormone control (Jump et al., 1994). Fasting can also cause a decrease in lipogenesis in adipose tissue as well as an increase in lipolysis. However, in the liver fasting results in a large increase in the number of fatty acids arriving and an increase in triglyceride synthesis occurring to provide energy for the body. This can result in a mild case of fatty liver (hepatosteatosis) (Kersten et al., 1999).

Hormonal. Insulin is widely acknowledged as having the most impactful role on lipogenesis with regards to a hormonal factor (Kersten, 2001). When the amount of glucose the animal intakes is increased, this results in an increase in glucose taken up by the adipose cell via recruitment of the glucose transporters to the plasma membrane, along with activating lipogenic and glycolytic enzymes via covalent modification (Kersten, 2001). The result of this is insulin powerfully stimulating lipogenesis. It does so by the binding of insulin to its receptor at the surface of the cell, resulting in the

activation of tyrosine kinase, triggering a large downstream effect due to tyrosine phosphorylation (Lane et al., 1990; Nakae and Accili, 1999; Kersten, 2001).

Growth hormone (GH) is another hormone that has a large impact on lipogenesis. It reduces lipogenesis, specifically in adipose tissue. In swine, porcine somatotropin (pST) has been shown to increase muscle growth by roughly 50%, while simultaneously decreasing adipose tissue accumulation up to 70% (Etherton, 2000). This decrease in lipogenesis occurs through two main methods. The first being the decrease of insulin sensitivity, resulting in the down regulation of fatty acid synthase expression in adipose tissue (Yin et al., 1998), though the details of the mechanism of this is unknown. The second possible way that GH decreases lipogenesis is by phosphorylating the transcription factors Stat5a and 5b, which have been shown to be essential for growth hormone function (Teglund et al., 1998). This has been shown to decrease fat accumulation in adipose tissue in a knock out model (Teglund et al., 1998).

Lastly, another hormone that has an unfavorable impact on lipogenesis is leptin. It stimulates the release of glycerol from adipocytes (Siegrist-Kaiser et al., 1997) by stimulating fatty acid oxidation as well as by inhibiting lipogenesis (Bai et al., 1996; Wang et al., 1999). The inhibiting of lipogenesis occurs due to the down regulation of the genes associated with fatty acid and triglyceride synthesis (Soukas et al., 2000). It has been shown that rats infused with leptin into the mediobasal hypothalamus have a reduction in white adipose tissue lipogenesis (Buettner et al., 2008).

Transcriptional Regulation. Many studies have shown that the effects nutrition and hormones have on the expression of lipogenic genes are mostly mediated by sterol

regulatory element-binding proteins (SREBPs), which are transcription factors that regulated the expression of genes related to fatty acid and cholesterol metabolism (Hua et al., 1993; Tontonoz et al., 1993; Yokoyama et al., 1993). SREBPs belong to a group of basic helix-loop-helix leucine zipper (bHLH-Zip) transcription factors, of which there are three types: SREBP-1a, SREBP-1c, and SREBP-2. Research has shown that SREBPs directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides and phospholipids, as well as the NADPH cofactor required to synthesize these molecules (Brown and Goldstein, 1997; Horton and Shimomura, 1999; Edwards et al., 2000; Sakakura et al., 2001; Horton, Goldstein and Brown, 2002). At normal levels of expression, SREBP-1c favors fatty acid synthesis while SREBP-2 favors cholesterol synthesis (Brown and Goldstein, 1997; Horton, Goldstein and Brown, 2002).

Lipolysis

Lipolysis, or the breakdown of fat, can be divided into two main categories – basal and demand lipolysis. In humans and rodents, basal lipolysis is determined by adipocyte size and dietary triacylglycerol (Kosteli et al., 2010; Magkos et al., 2016), while demand lipolysis is regulated hormonally in response to energy demands (Contreras et al., 2017). However, independent of the type of lipolysis, triacylglycerol (TAG) in the adipocyte lipid droplet are broken down by the action of three different lipases – adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoglyceride lipase (MGL). ATGL is the primary regulator of basal lipolysis in monogastric animals, acting exclusively on TAG (Contreras et al., 2017). HSL, which is

considered the rate-limiting factor for demand lipolysis (Miyoshi et al., 2008; Lass et al., 2011) hydrolyzes several lipid substrates, including TAG, diglycerides, monoglycerides and cholesterol esters (Lafontan and Langin, 2009). When it is activated by protein kinase A (PKA), HSL associates with fatty acid binding protein 4 (FABP4) to form a complex that localizes on the lipid droplet. MGL is what completes the lipolytic pathway, acting exclusively on monoglycerides.

Complete activation of the lipolytic process requires not only the activation of ATGL and HSL, but also the phosphorylation of perilipin-1 (PLIN1), a protein that protects the lipid droplet from the lipolytic activity of HSL. PLIN1 is phosphorylated by PKA to allow HSL interaction with TAG (Contreras et al., 2017).

Marbling

As an animal grows, it transitions from depositing muscle to depositing fat, starting with kidney, pelvic, heart (KPH) fat. This transition begins partly due to a decrease in growth hormone, which promotes protein synthesis. After a predetermined amount of KPH fat has been deposited, subcutaneous fat will begin to be deposited in larger amounts. This will eventually transition to intermuscular fat, and lastly intramuscular fat, what is commonly known as marbling. Marbling is important as it can play a part in tenderness, as well as palatability (Blumer, 1963; Pearson, 1966; Smith et al., 1985; Wheeler, Cundiff, and Koch, 1994).

MATERIALS AND METHODS

Data collected was in compliance with the Montana State University Agriculture Animal Care and Use Committee, number 2015-AA17.

Cattle Selection and Management

Fifteen steers from the Red Bluff Research Ranch were selected at weaning based on weight and date of calving, the parameters being ±22.7 kgs, with birthdates within a 14 day window. They were then relocated to the Montana State University Bozeman Area Research and Teaching Farm and placed in one pen together in a feedlot. At the start of the study, the steers weighed an average of 314.79 kgs. They were implanted once with Synovex One Feedlot in the back of the ear per Standard feedlot protocol and were weighed every 14 days once on full feed. They were fed an ad libitum diet of hay for 2 weeks to acclimate steers to pens, and were then started on a 6 week step up program to build up to the full ration, which was a diet of 75% shelled corn (table 1), 18% hay (table 2), and 7% of finisher pellet, produced by CHS (table 3), fed in a bunk daily. Bunks were checked twice daily to determine if more feed was needed based on the amount of feed left in the bunk since the last feeding. The pen was scraped and bedded as needed with straw, with feed and water checked twice daily. Steers had ad libitum access to feed and water.

Table 1. Shelled corn composition

	Dry Weight	Units	Reporting Limit
Protein (Crude)	9.55	%	0.20
Fat (Crude)	3.6	%	0.10
Fiber(acid detergent)	2.2	%	0.5
Ash	1.98	%	0.10
Total digestible nutrients	91.4	%	0.1
Net Energy (Lactation)	0.96	Mcal/lbs	0.01
Net Energy (Maint.)	1.01	Mcal/lbs	0.01
Net Energy (Gain)	0.67	Mcal/lbs	0.01
Digestible Energy	1.83	Mcal/lbs	0.01
Metabolizable Energy	1.72	Mcal/lbs	0.01
Sulfur (total)	0.1	%	0.01
Phosphorus	0.41	%	0.01
Potassium	0.46	%	0.01
Magnesium	0.16	%	0.01
Calcium	0.03	%	0.01
Sodium	n.d.	%	0.01
Iron	22	ppm	5.0
Manganese	6.7	ppm	1.0
Copper	2.8	ppm	1.0
Zinc	24.3	ppm	1.0

Table 2. Hay composition

	Dry Weight	Units	Reporting Limit
Protein (Crude)	17	%	0.20
Fat (Crude)	1.8	%	0.10
Fiber(acid detergent)	36.4	%	0.5
Ash	10.1	%	0.10
Total digestible nutrients	61.8	%	0.1
Net Energy (Lactation)	0.63	Mcal/lbs	0.01
Net Energy (Maint.)	0.62	Mcal/lbs	0.01
Net Energy (Gain)	0.35	Mcal/lbs	0.01
Digestible Energy	1.24	Mcal/lbs	0.01
Metabolizable Energy	1.14	Mcal/lbs	0.01
Sulfur (total)	0.24	%	0.01
Phosphorus	0.36	%	0.01
Potassium	2.17	%	0.01
Magnesium	0.25	%	0.01
Calcium	1.91	%	0.01
Sodium	0.07	%	0.01
Iron	476	ppm	5.0
Manganese	61.2	ppm	1.0
Copper	6.4	ppm	1.0
Zinc	92.5	ppm	1.0

Table 3. Concentrate pellet composition

	Dry Weight	Units	Reporting Limit
Protein (Crude)	42.2	%	0.20
Fat (Crude)	3.13	%	0.10
Fiber(acid detergent)	9.4	%	0.5
Ash	29.6	%	0.10
Total digestible nutrients	56.6	%	0.1
Net Energy (Lactation)	0.57	Mcal/lbs	0.01
Net Energy (Maint.)	0.56	Mcal/lbs	0.01
Net Energy (Gain)	0.32	Mcal/lbs	0.01
Digestible Energy	1.13	Mcal/lbs	0.01
Metabolizable Energy	0.99	Mcal/lbs	0.01
Sulfur (total)	0.64	%	0.01
Phosphorus	1.26	%	0.01
Potassium	2.31	%	0.01
Magnesium	0.33	%	0.01
Calcium	9.29	%	0.01
Sodium	1.19	%	0.01
Iron	214	ppm	5.0
Manganese	551	ppm	1.0
Copper	325	ppm	1.0
Zinc	1580	ppm	1.0

Carcass Data Collection

The steers were randomly allocated to one of three endpoints based off of growth rate and body weight, the endpoints being 431kg, 522kg, and 612kg. The aim of these weight-based endpoints was to produce carcasses grading Standard, Select, and Choice. The animals were harvested at Pioneer Meats in Big Timber, Montana. Carcasses were stored for 24hrs in a 40°C cooler prior to loin removal for use in determination of shear force. A stab probe was used to collect pH decline measurements at 1, 2, 3, 6, 12, and 24 hours postmortem. Intermuscular and subcutaneous adipose tissue samples were taken at time of harvest and were homogenized while longissimus thoracis muscle samples were

taken and were snap frozen for later gene expression analysis. Twenty-four hours after slaughter, loin samples were collected, cut into 2.54 centimeter steaks that were aged 1, 4, 7, 14, and 21 days postmortem. The steaks were used to evaluate shear force.

pH Decline

The decline of pH was measured in the longissimus thoracis, approximately 6cm from the hip bone, using an Accumet Portable AP110 and AP61 meter, Fisher Scientific Orion 8163BNWP electrode. The meter was standardized with buffers at pH 7 and 4. Measurements were taken hourly up to 8 hours, then at 12, and 24 hours postmortem.

Shear Force

Steaks were taken from a -20° C freezer and placed in a 2°C cooler approximately 24 hours prior to cooking. They were blotted, tagged, weighed, then had a single copper constantan thermocouple (OMEGA Engineering, INC, P.O. Box 4047, Stamford, Connecticut) placed in the center. They were then placed on an aluminum-covered broiler pan and placed in a conventional oven 10.16 cm below the heating element. Steaks were then cooked on broil until reaching an internal temperature of 35°C, at which point they were removed from the oven, rotated then returned to the oven and cooked on the other side until reaching an internal temperature of 70°C. They were then removed from the oven and set on a tray to cool and then placed into labeled Ziploc® bags to cool in a 2°C fridge. After cooling for a minimum of 45 minutes, steaks were taken from the cooler, blotted with towels and weighed. A minimum of 5 samples were taken parallel to the muscle fiber, resulting in square samples that 1.27 x 1.27cm. The samples were then

sheared using a TMS 30 Food Texturometer fitted with a Warner-Bratzler shear attachment. The average of the samples sheared was used for statistical analysis.

Gene Expression

Frozen muscle samples and homogenized intramuscular and intermuscular adipose tissue samples underwent RNA extraction using a Qiagen RNeasy Plus Universal Midi kit. Three grams of tissue were placed into 5mL of Qiazol Lysis Reagent in a 15mL tube. Samples were then homogenized using a Polytron tissue homogenizer for 20 seconds. After each sample, the polytron probe was cleaned with a three-step washing using detergent, water and RNase away. After adding 500µL of gDNA Eliminator, the samples were shaken for 15 seconds, 1 mL of chloroform was added and samples were shaken for an additional 15 seconds and then were allowed to rest for 3 minutes. The samples were then centrifuged at 3250 rotations per minute (rpm) at 4°C for 30 minutes. Approximately 3 mL of the clear aqueous solution was added into a new tube, which then had one volume of 70% ethanol added, and was then vortexed for 5 seconds. Four mL was then transferred into a spin column, and was then centrifuged for 5 minutes at 3250 rpms and 18°C, and the flow through was disposed of. The other 3-4 mL of sample was then placed in the spin column and was centrifuged for 5 minutes at 3250 rpms and 18°C, with the flow through being disposed of. Then the sample was washed by adding 4 mL of buffer RWT to the spin column, which was then centrifuged for 5 minutes at 3250 rpms at 18°C. The resulting flow through was disposed of, with 3 mL of RPE buffer being added to the spin column. The tube was then centrifuged again for 5 minutes at 3250 rpm at 18°C. Flow through was disposed of, and 2 mL of buffer was added to the spin

column. Tubes were spun for 5 minutes at 3250 rpm at 19° C. The spin column was then moved to a new tube, with 250mL of RNase free water added to the spin column. It was left to sit for 1 minute to elute before being placed back into the centrifuge for 5 minutes at 3250 rpm at 21°C. The flow-through was pipetted into an Eppendorf tubes and was stored in a -80°C freezer until pulled for quality analysis before being sent to Novogene, located in Sacramento, CA, for gene expression analysis.

Due to RNA degradation in some samples, we were unable to utilize samples from each adipose depot from each animal. The highest quality RNA sample from each animal was sent for further analysis, as well as sending random samples from each depot for 3 samples to verify that the adipose samples, regardless of depot, had a sample correlation of great than 0.8.

Quantification and Qualification

After arriving at Novogene, RNA degradation and contamination of the samples was monitored on 1% agarose gels. The purity was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, USA). Concentration of the RNA was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). Integrity of the RNA was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

Library Preparation

A total of 3 μg of RNA per sample was taken from total sample as input material for RNA sample preparation. Sequencing libraries were generated using NEBNext®

Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. Summarized briefly, mRNA was purified from total RNA with the use of poly-T oligo-attached magnetic beads. Fragmentation was done using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized with the use of random hexamer primer and M-MuLV Reverse Transcriptase (RNase H^-). Second strand cDNA synthesis was performed with the use of DNA Polymerase I and RNase H. Any remaining overhangs were altered to blunt ends using exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. Library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA) to select cDNA fragments preferentially 150~200 bp in length. Next, 3 µl USER Enzyme (NEB, USA) was added with size-selected, adaptor-ligated cDNA at 37 °C for 15 minutes followed by 5 minutes at 95 °C before PCR. PCR was then performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Lastly, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Clustering and Sequencing

Clustering of the index-coded samples was performed on a cBot Cluster Generation System with the use of HiSeq PE Cluster Kit cBot-HS (Illumina) according to the instructions from the manufacturer. After cluster generation, the library preparations

were sequenced on an Illumina Hiseq platform and 125 bp/150 bp paired-end reads were generated.

Quality Control

Raw data of fastq format were first processed through in-house perl scripts. During this step, clean data were obtained by removing reads that contained adapter, poly-N and low quality reads from the raw data. Simultaneously, Q20, Q30 and GC content with regards to the clean data were calculated. All downstream analyses were based on the clean data with high quality.

Read Mapping to the Reference Genome

Reference genome and gene model annotation files were downloaded directly from the genome website. Reference genome UMD 3.1 was utilized. An index of the reference genome was built using Bowtie v2.2.3 and paired-end clean reads were aligned to the reference genome using TopHat v2.0.12.

Quantification

The use of HTSeq v0.6.1 was employed to count the read numbers mapped to each gene. The FPKM, expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced, of each gene was then calculated based on the length of the gene and the reads count mapped to said gene.

Differential Expression Analysis

Prior to differential gene expression analysis, for each sequenced library the read counts were adjusted by edgeR program package through one scaling normalized factor.

Differential expression analysis of two conditions was performed using the DEGSeq R package (1.20.0). The p-values were adjusted using the Benjamini-Hochberg method. A corrected p-value of 0.005 and $\log_2$ (fold change) of 1 were set as the threshold for significantly differential expression.

GO and KEGG Enrichment Analysis

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with FDR corrected P-value of less than 0.05 were considered significantly enriched by differentially expressed genes. KEGG, a database resource for understanding high-level functions and utilities of the biological system, was used, along with KOBAS software, to test the statistical enrichment of differentially expressed genes in KEGG pathways.

PPI Analysis of Differentially Expressed Genes

Protein-Protein Interaction (PPI) analysis of differentially expressed genes was based on the STRING database, which contains known and predicted PPI values. For the species existing in the database, a network was constructed by extracting the target genes on the list from the database. If genes were not listed in the STRING database, Blastx (v2.2.28) was used to align the target gene sequences to the selected reference protein sequences, and the network was then built according to the known interaction of the selected reference species.

Novel Transcripts Prediction and Splicing Analysis

The Cufflinks v2.1.1 Reference Annotation Based Transcript (RABT) assembly method was used to construct and identify both known and novel transcripts from TopHat alignment results. Alternative splicing events were classified to 12 basic types by the software Asprofile v1.0. The number of alternative splicing events in each sample was estimated.

SNP Analysis

Picard-tools v1.96 and samtools v0.1.18 were used to sort, mark duplicated reads and reorder the bam alignment results of each sample. GATK2 (v3.2) software was used to perform SNP calling.

RESULTS AND DISCUSSION

Average Daily Gain

As is shown in table 4, animals that graded Choice and Select tended to have a higher average daily gain (ADG) compared to those that graded Standard. The ADG of Choice, Select and Standard were 1.85 kg, 1.81 kg and 1.52 kg respectively. There was significant differences in gain between Choice and Standard (P=0.02874), and Select and Standard (P=0.006161), but not significant between Choice and Select (P=0.656).

Table 4. Animal ID, live weight, ADG and quality grading

Animal ID	Start (kg)	End (kg)	ADG (kg)	Quality
40	293.85	427.50	1.59	Choice
43	349.20	618.30	1.73	Choice
44	304.20	641.93	2.16	Choice
53	312.75	590.63	1.78	Choice
54	296.10	481.05	1.76	Select
55	301.50	438.30	1.63	Standard
64	299.25	439.20	1.67	Standard
69	310.50	505.80	1.86	Select
74	333.00	615.38	1.81	Choice
77	314.55	506.25	1.83	Select
81	308.70	625.05	2.03	Choice
86	319.95	430.65	1.32	Standard
89	318.15	504.45	1.77	Select
94	313.20	503.55	1.81	Select
95	314.55	436.95	1.46	Standard

Shear Force

Carcasses that graded Choice tended to have a lower shear force value than carcasses that graded Select and Standard (table 5). It is important to note that Choice steaks aged 24 hours and 7 days were less tender than Select steaks. This could be due to error when samples were cut, placement on the TMS 30 Food Texturometer, or due to difference in aging process between the cohorts. Our results support studies done previously by others (Hiner and Hankins, 1950; Simone, Carroll & Clegg 1958; Zinn, Durham and Hedrick, 1970). Shear force values between Choice and Select were only significant when steaks were aged 24 hours (P=0.02196). When comparing Choice to Standard steaks, shear force values were significant at fourteen days only (P=0.0009601), though this difference seems unnaturally large. This could be due to having a large variation in shear force values in the Choice as well as the Standard steaks. When Select and Standard shear force values were compared, there were significant differences at 24 hours (P=0.03168) and seven days (P=0.0457) of age.

Table 5. Average shear force values based on age of steak

Age	Choice (N)	Select (N)	Standard (N)
24hr	114.68	85.81	124.52
3 Day	81.49	88.95	114.31
7 Day	80.94	77.45	105.50
14 Day	64.77	73.84	92.06
21 Day	61.57	63.91	87.69

N= 9.81 kg

Gene expression

Four adipose samples required re-extraction and were resent to Novogene for analysis. As is evident in table 6, there was a decrease in the number of read counts between raw and clean data, a lower Q30 than Q20, as well as a higher percent GC content across all cohorts, in both adipose and muscle tissue.

Table 6. Gene expression reads, quality of reads, G and C content in reads.

	Adipose				
	Raw	Clean	Q20 (%)	Q30 (%)	GC Content (%)
Standard	52994544	51102307.5	96.59	91.53	54.05
Select	53655296	52008329.33	96.61	91.50	54.26
Choice	68918968.33	66800801.33	97.10	92.70	54.63

	Muscle				
	Raw	Clean	Q20 (%)	Q30 (%)	GC Content (%)
Standard	51324601.5	49558730	96.19	90.55	52.86
Select	57170688.8	55292644.4	96.46	91.12	54.16
Choice	48957502.33	46709699	96.62	91.41	54.79

Raw Reads: the original sequencing reads counts
Clean Reads: number of reads after filtering
Clean Bases: clean reads number multiply read length, saved in G unit
Q20: percentages of bases whose correct base recognition rates are greater than 99% in total bases
Q30: percentages of bases whose correct base recognition rates are greater than 99.9% in total bases
GC content: percentages of G and C in total base

The percent of reads mapped was well above the recommended 70%, as is seen in tables 7a and 7b. Few of the genes mapped were able to be mapped to multiple sites on the reference genome, there was almost equal number of reads that were mapped to the '+', or sense strand, as there were mapped to the '-', or antisense, strand. When looking at spliced reads, which is the reads that can be segmented and mapped to two exons, versus

non-spliced reads, reads mapped to a single exon, it is evident that there are more non-spliced reads in all instances.

Alternative Splicing

Alternative splicing (AS) is considered a universal gene regulation mechanism that occurs in most eukaryotes. In eukaryotic animals, DNA has exonic and intronic regions: the exonic encodes a part of the final mRNA product while the intronic regions do not code for protein synthesis and thereby are removed by spliceosomes during RNA synthesis. There are five major AS events (see figure 1): skipped exon (SE) which results in the exon being removed from the transcript along with the flanking introns; alternative 5' splice site (A5SS) and alternative 3' splice site (A3SS) which both result with the recognition of two or more splice sites at one end of an exon; mutually exclusive exons (MXE), when one of two exons is retained in mRNAs after splicing, but not both; retained intron (RI), which occurs when an intron has failed to be removed from the sequence. Of the types of AS listed above, the skipping of exons is the most common, making up roughly ~30% of AS events (Wang et al., 2015). This was evident in this study, with 87.6% of all AS events being SE, as well as having more significant AS events (table 8).

Table 7a. Adipose mapping overview

	Standard	Select	Choice
Total mapped	42957972.00	44594760.00	56630927.83
Total mapped (%)	84.34	85.78	84.85
Multiple mapped	527412.50	458310.33	652230.83
Multiple mapped (%)	1.03	0.88	0.96
Reads map to '+'	21187480.50	22040259.00	27936887.33
Reads map to '-'	21243079.00	22096190.67	28041809.67
Non-splice reads	24896146.75	25234077.33	31658393.00
Splice reads	17534412.75	18902372.33	24320304.00

Table 7b. Muscle mapping overview

	Standard	Select	Choice
Total mapped	42160277.00	46898632.40	39590712.17
Total mapped (%)	85.05	84.71	84.63
Multiple mapped	666079.75	602578.40	697594.17
Multiple mapped (%)	1.33	1.08	1.47
Reads map to '+'	20768332.00	23176354.00	19472306.83
Reads map to '-'	20725865.25	23119700.00	19420811.17
Non-splice reads	22823698.50	25348294.20	19971267.67
Splice reads	18670498.75	20947759.80	18921850.33

Figure 1. Diagram of alternative splicing events

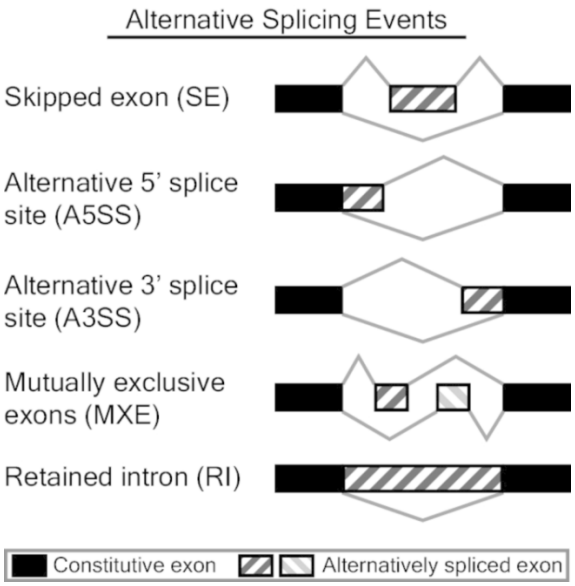


Table 8. Statistics of AS events

AS type	NumEvents.JC .only	SigEvents.JC. only	NumEvents.JC +readsOnTarget	SigEvents.JC +readsOnTarget
SE	19346	13 (8:5)	19354	11 (7:4)
MXE	2616	4 (1:3)	2617	2 (1:1)
A5SS	24	0 (0:0)	24	0 (0:0)
A3SS	55	0 (0:0)	55	0 (0:0)
RI	44	0 (0:0)	44	0 (0:0)

NumEvents.JC.only: the total number of AS events, with only reads span splicing junctions taken into account.
SigEvents.JC.only: the total number of differential AS events, with only reads span splicing junctions taken into account(up:down).
NumEvents.JC+readsOnTarget: the total number of AS events, with both reads span splicing junctions and reads on target exons taken into account.
SigEvents.JC+readsOnTarget: the total number of differential AS events, with both reads span splicing junctions and reads on target exons taken into account.

Single Nucleotide Polymorphism and InDel

A single nucleotide polymorphism (SNP) is a variation in the sequence of DNA that is commonly occurring within a population where a single nucleotide has been changed, resulting in it to differ between members of a species or paired chromosomes. InDel on the other hand are small fragments, one or more nucleotide, that have been inserted or deleted. There were more instances of SNP variants than there were of InDels, though the percent of occurrence was similar between both adipose and muscle tissues with regards to SNPs and InDels (table 9). These values are consistent with previous observations in the bovine (Pruitt et al., 2014).

Table 9. Average number of SNP and InDel

	Adipose Tissue			Muscle Tissue		
	Standard	Select	Choice	Standard	Select	Choice
SNP	147159.25	164464.67	162881.83	135262	117548.8	104262.67
SNP%	85.6%	85.9%	85.9%	85.9%	85.4%	85.7%
InDel	24742.5	26954.333	26774.833	22258	20071.8	17463
InDel%	14.4%	14.1%	14.1%	14.1%	14.6%	14.3%

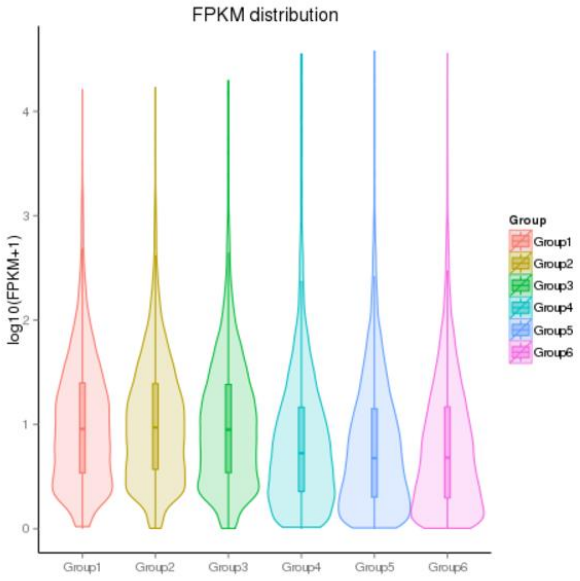
Novel Transcripts

A novel transcript is a transcript that is either newly discovered or its function is unknown. Between all cohorts in this study, there were a total of 22,624 novel transcripts recorded. Of those genes, all were located on the exon. A total of 589,747 novel gene isoforms were found between all cohorts. A novel gene isoform are genes that form due to mRNAs that are produced from the same site on a chromosome but that differ in transcription state sites, protein coding DNA sequences, or untranslated regions. These changes potentially alter the function of the gene. There were 12,828 genes found that varied in location in the transcriptome submitted versus the reference genome. A possible explanation for the high amount of novel transcripts could be due to the annotation of the bovine genome being incomplete.

Expression Quantification

The level of gene expression is measured by transcript abundance – the greater the abundance, the higher the gene expression level. This is estimated using the expected number of fragments per kilobase of transcript sequenced per million base pairs sequenced (FPKM). This method takes into account the effects of both sequencing depth and gene length of fragments (Trapnell et al., 2010). Adipose tissue from carcasses grading Standard, Select and Choice had comparable FPKM distribution. Similarly, muscle tissue from carcasses grading Standard, Select and Choice had similar distribution patterns. This was to be expected due to the nature of the samples (figure 2).

Figure 2. FPKM density

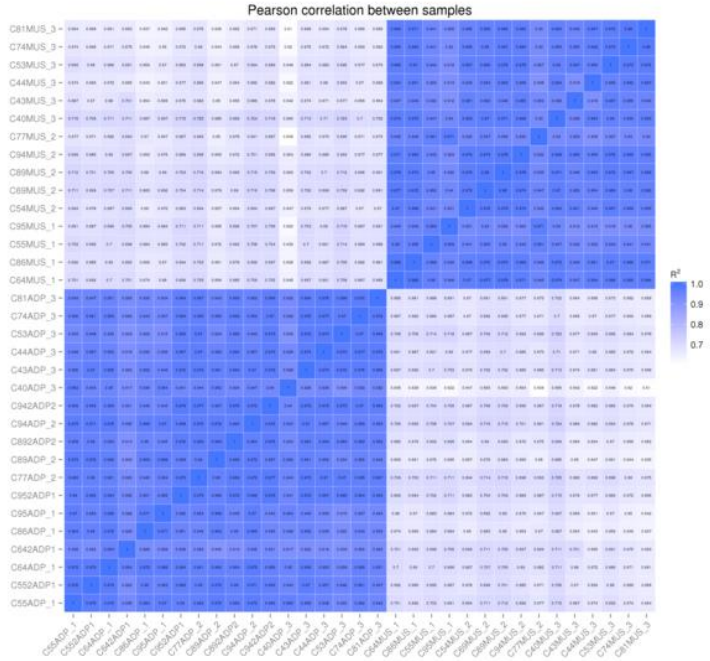


Density is depicted on the x-axis

RNA-Seq Correlation

Pearson correlation shows the similarity between samples, as well as the repeatability of the experiment. The closer the correlation coefficient is to 1, the higher the resemblance between samples. The Encyclopedia of DNA Elements recommends the square of the Pearson correlation coefficient be larger than 0.92 under ideal conditions, though for this study, the correlation should be higher than 0.8 which is the threshold for replicates to be used in a multiple comparison experiment. As is evident in the Pearson correlation for this study (figure 3), there was high correlation within muscle samples, as well as within adipose samples. There was little correlation between adipose and muscle samples.

Figure 3. Pearson correlation between samples



Differentially Expressed Genes (DEG)

With animals grading different qualities, it is to be expected that genes would be expressed differently between quality grades. This is exactly what was seen. When comparing Select to Standard adipose tissue samples, four genes were down regulated while 29 genes were up regulated. Of those genes, only one down regulated gene was significantly differentially expressed, which was the major histocompatibility complex, class II, DM beta (table 10). We did not expect to see any major histocompatibility complexes being expressed in adipose tissue as these genes are typically found in antigen presenting tissue. However, our samples were whole tissue which includes the resident and transient immune cells present in that tissue. Of the genes being up regulated, only two were significantly differentially expressed that were related to adipose tissue:

peroxisome proliferator activated receptor alpha (PPARα) and protein kinase AMP-activated non-catalytic subunit beta 2 (PRKAB2) (table 11).

When comparing the Select to Choice adipose tissue samples, eight genes were seen to be down regulated, while 15 were being up regulated. Of these genes, only two were being significantly differentially expressed as they were down regulated: adenylate cyclase type 5 (ADCY5) and leptin (LEP) (table 12).

The comparison of Choice to Standard adipose tissue samples contained the most differentially expressed genes, with 49 genes down regulated and 113 genes up regulated. Of the genes being down regulated, seven were significantly altered in their gene expression levels: eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1); hedgehog interacting protein (HHIP); reelin precursor (RELN), nerve growth factor (NGF); major histocompatibility complex, class II, DQA2 (BOLA-DQA2); major histocompatibility complex, class II, DQ beta (BOLA-DQB); major histocompatibility complex, class II, DQ alpha 5 (BOLA-DQA5) (table 13). Concerning the genes being significantly up regulated in the Choice to Standard adipose tissue, there was a total of nine: calcium binding protein 39 like (CAB39L); fibroblast growth factor 1 (FGF1); glutamate ionotropic receptor NMDA type subunit 1 (GRIN1); leptin; hexokinase 2 (HK2); tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma (YWHAG); Stearoyl-CoA desaturase (SCD1); ELOVL fatty acid elongase 5 (ELVOL5) (table 14).

Of the Standard to Choice muscle tissue samples, a total of 15 genes were down regulated, while 20 were up regulated. Of the genes that were down regulated, only

insulin receptor substrate 1 (IRS1) seemed to have an impact on the development of the muscle (table 15), while BOLA-DMB and BOLA-DQA2 were significantly up regulated (table 16). There were differentially expressed genes between muscle from carcasses that graded Standard and those grading Select. When comparing the Select and Choice muscle groups, a total of four genes were being up regulated, with only one being down regulated, none of which had an impact on muscle development or deposition.

Table 10. Select to Standard significantly down regulated adipose tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000021077	0	446.4814534	3.70E-08	BOLA-DMB	Major histocompatibility complex, class II, DM beta

Table 11. Select to Standard significantly up regulated adipose tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000008063	466.1987041	193.6748103	2.47E-05	PPARA	peroxisome proliferator activated receptor alpha
ENSBTAG00000014387	52.33053568	13.48899581	2.19E-05	PRKAB2	protein kinase AMP-activated non-catalytic subunit beta 2

Table 12. Select to Choice significantly down regulated adipose tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000018777	818.9029568	2158.209098	6.08E-07	ADCY5	adenylate cyclase type 5
ENSBTAG00000014911	2371.16379	8660.135631	2.46E-05	LEP	leptin

Table 13. Choice to Standard significantly down regulated adipose tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000027654	2192.044386	5928.113361	7.97E-05	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1
ENSBTAG00000016071	17.35247576	52.08435641	1.95E-04	HHIP	hedgehog interacting protein
ENSBTAG00000003658	70.73350439	166.1783114	4.50E-05	RELN	reelin precursor
ENSBTAG00000007446	25.43454316	71.64164715	8.45E-05	NGF	nerve growth factor
ENSBTAG00000009656	45.94515343	133.4541166	5.51E-06	BOLA-DQA2	Major histocompatibility complex, class II, DQ alpha 2
ENSBTAG00000021077	0.146795505	480.3474362	1.40E-08	BOLA-DQB	Major histocompatibility complex, class II, DQ beta
ENSBTAG00000038128	101.0001041	519.8998552	1.36E-06	BOLA-DQA5	Major histocompatibility complex, class II, DQ alpha 5

Table 14. Choice to Standard significantly up regulated adipose tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000034222	190.0880086	83.56420309	4.76E-05	CAB39L	calcium binding protein 39 like
ENSBTAG00000005198	506.3863588	145.3035765	2.62E-07	FGF1	Fibroblast growth factor 1
ENSBTAG00000047202	126.5940365	49.01673285	8.74E-06	GRIN1	glutamate ionotropic receptor NMDA type subunit 1
ENSBTAG00000014911	8938.900072	3504.577478	2.96E-04	LEP	leptin
ENSBTAG00000013108	746.2792459	163.4152755	9.91E-05	HK2	hexokinase 2
ENSBTAG00000004077	8903.965131	4647.780626	3.26E-04	YWHAG	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma
ENSBTAG00000017567	41420.41051	11910.59833	1.48E-06	ACC1	Acetyl-CoA carboxylase alpha
ENSBTAG00000045728	113910.7939	31860.2529	1.15E-04	SCD1	Stearoyl-CoA desaturase
ENSBTAG00000003359	18780.52553	6929.674559	4.39E-06	ELOVL5	ELOVL fatty acid elongase 5

Table 15. Standard to Choice significantly down regulated muscle tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000021308	600.7675	1307.075	3.38E-05	IRS1	insulin receptor substrate 1

Table 16. Standard to Choice significantly up regulated muscle tissue DEGs

Gene code	Read count	Read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000009656	25.03987	3.93732	7.16E-07	BOLA-DQA2	Major histocompatibility complex, class II, DQ alpha 2
ENSBTAG00000012451	186.654	72.58464	5.64E-05	BOLA-DMB	Major histocompatibility complex, class II, DM beta

GO Enrichment Analysis

Gene Ontology (GO) is a technique for interpreting sets of genes making use of the Gene Ontology system of classification, in which genes are assigned to a set of predefined bins depending on their functional characteristics. GO provides a system for hierarchically classifying genes or gene products to terms organized in a graph structure called an ontology. The terms are grouped into three categories: molecular function, which describes the molecular activity of a gene; biological process, describing the larger cellular or physiological role carried out by the gene which are coordinated with other genes; and cellular component, which describes the location in the cell where the gene product executes its function.

When analyzing GO results, there were no lipid related genes being down regulated when comparing adipose tissue samples from carcasses that graded Select and Standard. This is to be expected as the select carcasses are higher quality with more lipid deposition. However, there were multiple adipose-related genes being up regulated (table 17), such as the negative regulation of JAK-STAT cascade, the negative regulation of lipid storage, and the negative regulation of sequestering of triglyceride. These three processes have a negative impact on adipose tissue development as well as deposition.

Conversely, there was a large number of lipid related genes being down regulated when comparing adipose tissue samples from Select and Choice carcasses (table 18). Interestingly, the processes that are being down regulated in Select adipose tissue carcasses are ones that promote lipogenesis such as – adipose tissue development, positive regulation of JAK-STAT cascade, positive regulation of fatty acid biosynthetic

process, positive regulation of fatty acid metabolic process, positive regulation of lipid biosynthetic process. This would suggest that less adipose deposition is taking place in Select animals when compared to Choice animals.

With the comparison of adipose tissue from carcasses that graded Choice and Standard, a larger number of genes were seen being significantly up regulated in the Choice carcasses (table 19), all of which assist with the deposition of adipose tissue. The processes of importance are fatty acid biosynthetic process, fatty acid elongation, monounsaturated fatty acid, fatty acid metabolic process, adipose tissue development, positive regulation of fatty acid biosynthetic process, and the positive regulation of fatty acid metabolic process. Interestingly, there was one process of importance that had one gene in the process being down regulated, fatty-acyl-CoA synthase activity, which is essential to the production of fatty acids.

When analyzing muscle samples from carcasses that graded Standard and Select, there were not enough difference in the genes to utilize GO enrichment. When comparing Select to Choice muscle samples, there were a few enriched up regulated processes, but none directly related to muscle development. However, this was not the case when comparing Standard and Choice muscle samples (table 20), where there were processes being both up and down regulated, though only one was being significantly altered. This could be due to the fact that Standard carcasses are still growing and have not yet deposited much adipose tissue, whereas Choice carcasses are more developed and are focused on depositing adipose tissue, resulting in more adipose tissue pathways being active than muscle.

Table 17. GO enrichment of Select to Standard adipose tissue

GO accession	Description	Term type	Over represented P-value	Corrected P-value	DEG item	DEG list	Bg item	Up	Down
GO:0006629	lipid metabolic process	biological	0.042408	1	4	27	956	4	0
GO:0010887	negative regulation of cholesterol storage	biological	0.005227	1	1	27	4	1	0
GO:0046426	negative regulation of JAK-STAT cascade	biological	0.056617	1	1	27	45	1	0
GO:0010888	negative regulation of lipid storage	biological	0.016879	1	1	27	12	1	0
GO:0010891	negative regulation of sequestering of triglyceride	biological	0.006795	1	1	27	5	1	0

Table 18. GO enrichment of Select to Choice adipose tissue

GO accession	Description	Term type	Over represented P-value	Corrected P-value	DEG item	DEG list	Bg item	Up	Down
GO:0060612	adipose tissue development	biological	0.031208	1	1	20	28	0	1
GO:0046427	positive regulation of JAK-STAT cascade	biological	0.044895	1	1	20	48	0	1
GO:0045723	positive regulation of fatty acid biosynthetic process	biological	0.012979	1	1	20	14	0	1
GO:0045923	positive regulation of fatty acid metabolic process	biological	0.025569	1	1	20	26	0	1
GO:0046889	positive regulation of lipid biosynthetic process	biological	0.042763	1	1	20	44	0	1

Table 19. GO enrichment of Choice to Standard adipose tissue

GO accession	Description	Term type	Over represented P-Value	Corrected P-Value	DEG item	DEG list	Bg item	Up	Down
GO:0006633	fatty acid biosynthetic process	biological	0.000142	0.39674	6	143	104	5	1
GO:0034625	fatty acid elongation, monounsaturated fatty acid	biological	0.047926	1	1	143	7	1	0
GO:0006631	fatty acid metabolic process	biological	0.014188	1	6	143	254	5	1
GO:0060612	adipose tissue development	biological	0.023793	1	2	143	28	2	0
GO:0045723	positive regulation of fatty acid biosynthetic process	biological	0.004313	1	2	143	14	2	0
GO:0045923	positive regulation of fatty acid metabolic process	biological	0.016167	1	2	143	26	2	0
GO:0004321	fatty-acyl-CoA synthase activity	molecular	0.032504	1	1	143	4	0	1

Table 20. GO enrichment of Standard to Choice muscle tissue

GO accession	Description	Term type	Over represented P-value	Corrected P-value	DEG item	DEG list	Bg item	Up	Down
GO:0007015	actin filament organization	biological	0.35551	1	1	26	305	1	0
GO:0030029	actin filament-based process	biological	0.563	1	1	26	556	1	0
GO:0001578	microtubule bundle formation	biological	0.005679	1	2	26	75	1	1
GO:0000226	microtubule cytoskeleton organization	biological	0.13508	1	2	26	433	1	1
GO:0046785	microtubule polymerization	biological	0.072982	1	1	26	51	0	1

KEGG Pathway Enrichment

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of manually curated databases dealing with genomes, biological pathways, diseases, drugs and chemical substances. It is used for bioinformatics research and education, including but not limited to data analysis in genomics, metagenomics, and metabolomics. Pathway enrichment analysis identifies significantly enriched metabolic pathways of signals transduction pathways associated with DEGs compared with the whole genome background. The formula used is below (figure 4), where N is the number of all genes with a KEGG annotation, n is the number of DEGs in N, M is the number of all genes annotated to specific pathways, and m is the number of DEGs in M.

Figure 4. KEGG equation

$$p = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

Of the pathways highlighted via KEGG, ones of importance when comparing adipose tissue samples from Select and Standard carcasses were the AMPK signaling pathway (figure 5) due to the up regulation of PRKAB2, which can lead to the inhibition of fatty acid biosynthesis, the biosynthesis of unsaturated fatty acids, the mTOR signaling pathway, starch and sucrose metabolism, protein synthesis, the regulation of autophagy, and cell cycle DNA replication, while potentially promoting glycolysis and gluconeogenesis. The adipocytokine signaling pathway (figure 6) was relevant due to the

up regulation of PRKAB2 and PPARα which can lead to the inhibition of insulin resistance and down regulation of gluconeogenesis, while theoretically promoting a decrease in food intake, peroxisome proliferation, fatty acid metabolism and an increase in energy expenditure. The insulin signaling pathway (figure 7) was altered due to the up regulation of PRKAB2 which can cause the inhibition of lipogenesis, fatty acid biosynthesis and lipid homeostasis. The cAMP (figure 8) due to the up regulation of PPARα which has the potential to promote the stimulation of fatty acid β-oxidation. PPAR signaling pathway (figure 9) due to the regulation of PPARα, which can promote fatty acid degradation, bile acid biosynthesis, the synthesis and degradation of ketone bodies, glycerophospholipid metabolism, lipid metabolism and adipocyte differentiation.

With regards to the pathways altered between adipose tissue samples from Select and Choice carcasses, the adipocytokine signaling pathway (figure 10) was affected due to the down regulation of leptin, which can cause the inhibition of the down regulation of glucose uptake, down regulation of food intake, fatty acid metabolism, and peroxisome proliferation, while promoting an increase in energy expenditure, inhibition of gluconeogenesis and insulin resistance. The AMPK signaling pathway (figure 11) with the down regulation of leptin can cause an inhibition of glycolysis and gluconeogenesis, while being able to promote cell cycle DNA replication, the mTOR signaling pathway, fatty acid biosynthesis, biosynthesis of unsaturated fatty acids, regulation of autophagy, and starch and sucrose metabolism. The JAK-STAT signaling pathway (figure 12), with the down regulation of leptin can cause the inhibition of apoptosis, PI3K-Akt signaling pathway, cell cycle progression and inhibition, lipid metabolism and the MAPK signaling

pathway. The cAMP signaling pathway (figure 13), by having ADCY5 being down regulated, can stimulate the inhibition of fatty acid degradation, pancreatic secretion, bile secretion, long-term potentiation, cardiac muscle, the PI3K-Akt signaling pathway as well as the Rap1 signaling pathway, with the potential to promote apoptosis;. The Rap1 signaling pathway (figure 14) was altered due to the down regulation of ADCY5, which can inhibit the MAPK and PI3K-Akt signaling pathways, as well as causing cell adhesion, migration and polarity to be inhibited, in contrast, the regulation of the actin cytoskeleton, adherens junction, focal adhesion and defective angiogenesis can be promoted.

Of the adipose groups, there were the most differences with regards to adipose tissue samples from Choice and Standard carcasses, which was not surprising as they were the farthest apart with regards to maturity. Specific pathways of importance were the adipocytokine signaling pathway (figure15), which due to the up regulation of leptin can cause the inhibition of insulin resistance and the down regulation of gluconeogenesis, and with the ability to promote a decrease in food intake, inhibition of glucose uptake, and increase in energy expenditure, peroxisome proliferation and fatty acid metabolism. The AMPK signaling pathway (figure 16) had an up regulation of leptin, CAB39L, ACC1 and SCD1, with a down regulation of EIF4EBP1, resulting in the possibility of cell cycle DNA replication, starch and sucrose metabolism, fatty acid biosynthesis, biosynthesis of unsaturated fatty acids and the mTOR pathway being inhibited while glycolysis and gluconeogenesis, fatty acid biosynthesis and the biosynthesis of unsaturated fatty acids could be promoted. The cAMP pathway (figure 17) was altered

due to the up regulation of GRIN1 and the down regulation of HHIP, which resulted in the inhibition of the down regulation of the Hedgehog signaling pathway. The fatty acid biosynthesis pathway (figure 18), which due to the up regulation of ACC1 makes it possible for pyruvate metabolism, β-alanine metabolism, lipoic acid metabolism, fatty acid degradation, glycerolipid metabolism, glycerophospholipid metabolism and fatty acid elongation to be promoted. There was an up regulation of ELOVL5 in fatty acid elongation cycle (figure 19), which is known to cause a potential promotion of cutin, suberine and wax biosynthesis but as this role is unlikely in the bovine, it may have another function. Both glycolysis and gluconeogenesis (figure 20) were altered due to the up regulation of HK2 can cause a promotion of the pentose phosphate pathway, citrate cycle, pyruvate metabolism and propanoate metabolism. The insulin signaling pathway (figure 21) had an up regulation of ACC1, HK2, and EIF4E, and a down regulation of EIF4EBP1, which can cause the inhibition of protein synthesis and the promotion of lipogenesis and glycolysis. The down regulation of leptin in the JAK-STAT signaling pathway (figure 22) can cause apoptosis, the PI3K-Akt signaling pathway, cell cycle progression and inhibition, lipid metabolism and MAPK signaling pathway to be promoted. PI3K-Akt signaling pathway (figure 23) had a down regulation of NGF, RELN, and EIF4EBP1, while EIF4E, YWHAG and FGF1 were up regulated, which can cause protein synthesis, cell cycle progression and survival and apoptosis to be inhibited, with actin reorganization, glucose uptake, glycolysis, angiogenesis, and cell proliferation being potentially promoted. SCD1 was up regulated in the PPARα signaling pathway (figure 24), which can cause a promotion of fatty acid degradation, bile acid biosynthesis,

the synthesis and degradation of ketone bodies, lipid metabolism and glycerophospholipid metabolism. The Rap1 signaling pathway (figure 25) showed a down regulation of NGF, while GRIN1 and FGF1 were up regulated. This can cause an inhibition of defective angiogenesis, focal adhesion and the regulation of actin cytoskeleton, while potentially promoting adherens junction, cell adhesion, migration and polarity, as well as the MAPK and PI3K-Akt signaling pathways.

When looking at muscle tissue samples from Select and Standard carcasses, similar to the GO analysis, there were not enough differences to be able to compute an accurate KEGG analysis. While muscle tissue samples from Select to Choice carcasses contained pathways that were expressed differently, none were relevant to muscle development, such as β-alanine metabolism. When relating muscle tissue samples from Standard to Choice carcasses however, quite a few pathways were being impacted by differentially expressed genes. The first is the AMPK signaling pathway (figure 26). IRS1 was down regulated, causing the potential inhibition of cell growth and protein synthesis, autophagy and the mTOR signaling pathway can also be promoted. The IRS1 gene was also down regulated in the FOXO signaling pathway (figure 27), resulting in possible promotion of cell cycle regulation, apoptosis, regulation of autophagy, glycolysis and gluconeogenesis, muscle atrophy, and immune-regulation. Similarly, the mTOR pathway (figure 28) also had IRS1 down regulated, potentially causing the regulation of actin cytoskeleton and autophagy to be inhibited, with lipolysis, lipid biosynthesis, microtubule and protein synthesis to be promoted. Lastly, the PI3K-Akt signaling pathway (figure 29) was also potentially impacted by the down regulation of

IRS1, making it possible for glycolysis, gluconeogenesis, and cell cycle survival and progression to be inhibited while a possibility of apoptosis, protein synthesis and actin reorganization to be promoted.

Major histocompatibility complexes (MHC) were seen to be altered in the Select to Standard adipose tissue with the down regulation of BOLA-DQB, in Choice to Standard adipose tissue with the down regulation of BOLA-DQA2, BOLA-DQA5, and BOLA-DQB, while BOLA-DQA2 and BOLA-DQM were up regulated in the Standard to Choice muscle tissue, specifically with regards to the asthma (figure 30) and tuberculosis (figure 31) pathways being examples of where these genes play a potential role if not in the bovine in these tissues. The exact reason for this is unknown, as MHC's are not expected to be seen in adipose or muscle tissue cell types. They are known to be expressed by immune cells such as macrophages. A possible explanation for this could be that, for a reason yet unknown, macrophages are migrating from adipose tissue to muscle tissue. A study by Khan et al., (2015) showed instances where, in obese humans, MHC's were found to be increased in skeletal muscle of obese humans when compared to lean controls. It could be that MHC's are passing though adipose tissue to reach the muscle. Why this is occurring, however, is unknown and is worthy of future research.

Figure 5. Select to Standard adipose tissue AMPK signaling pathway

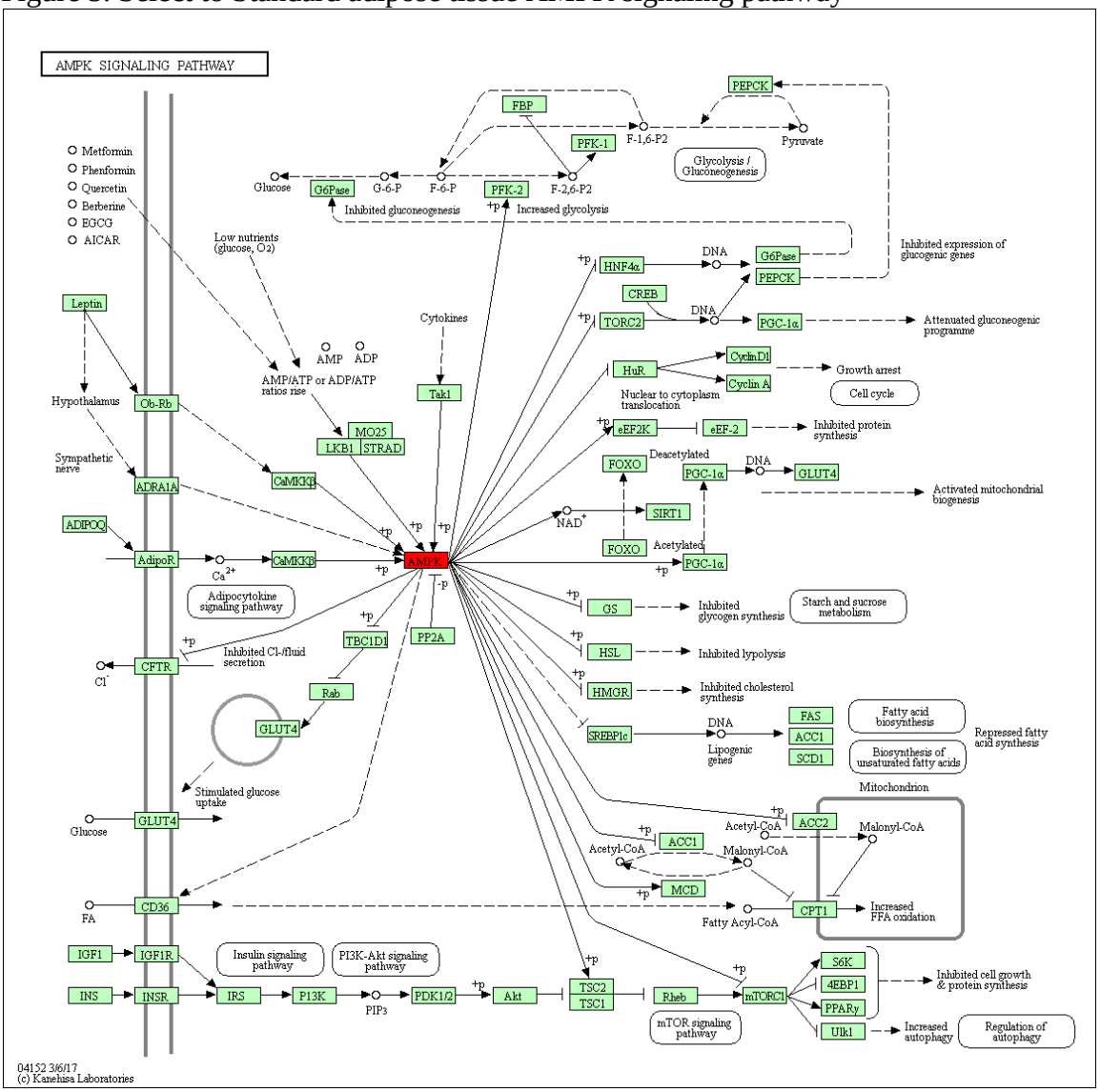


Figure 6. Select to Standard adipose tissue adipocytokine signaling pathway

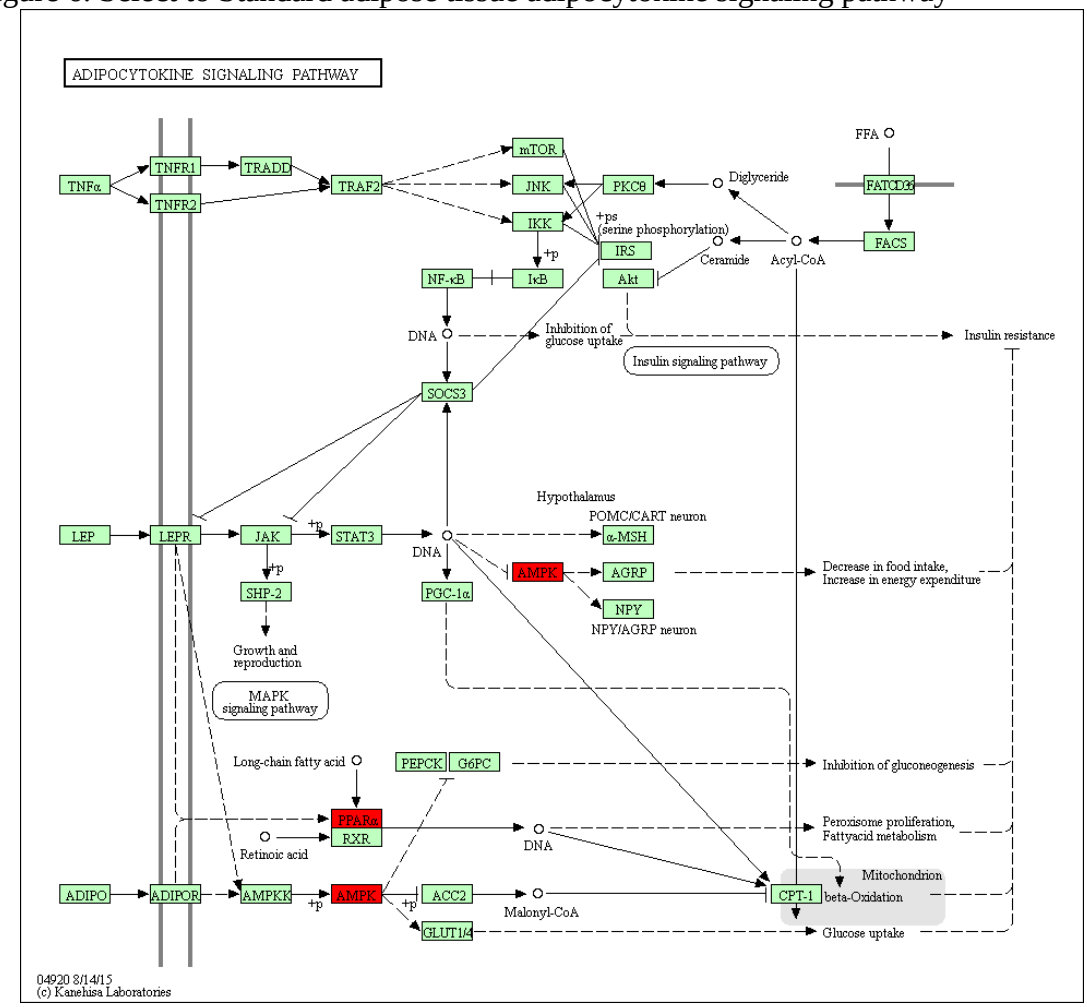


Figure 7. Select to Standard adipose tissue insulin signaling pathway

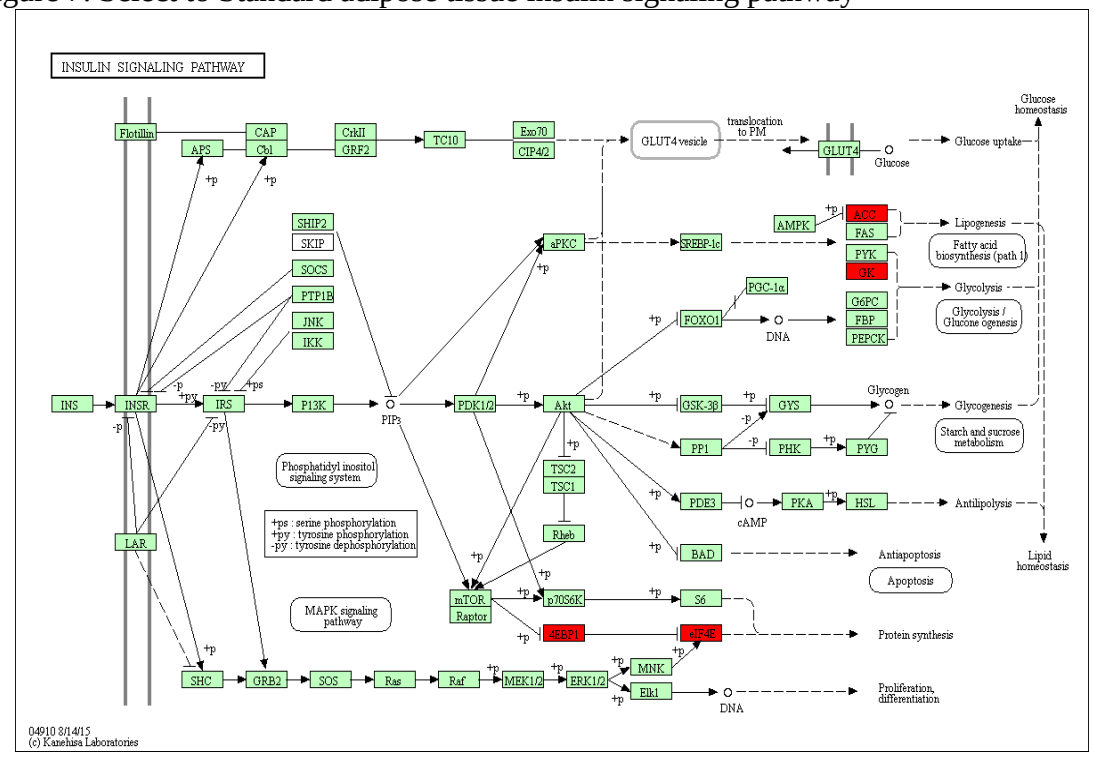


Figure 8. Select to Standard adipose tissue cAMP signaling pathway

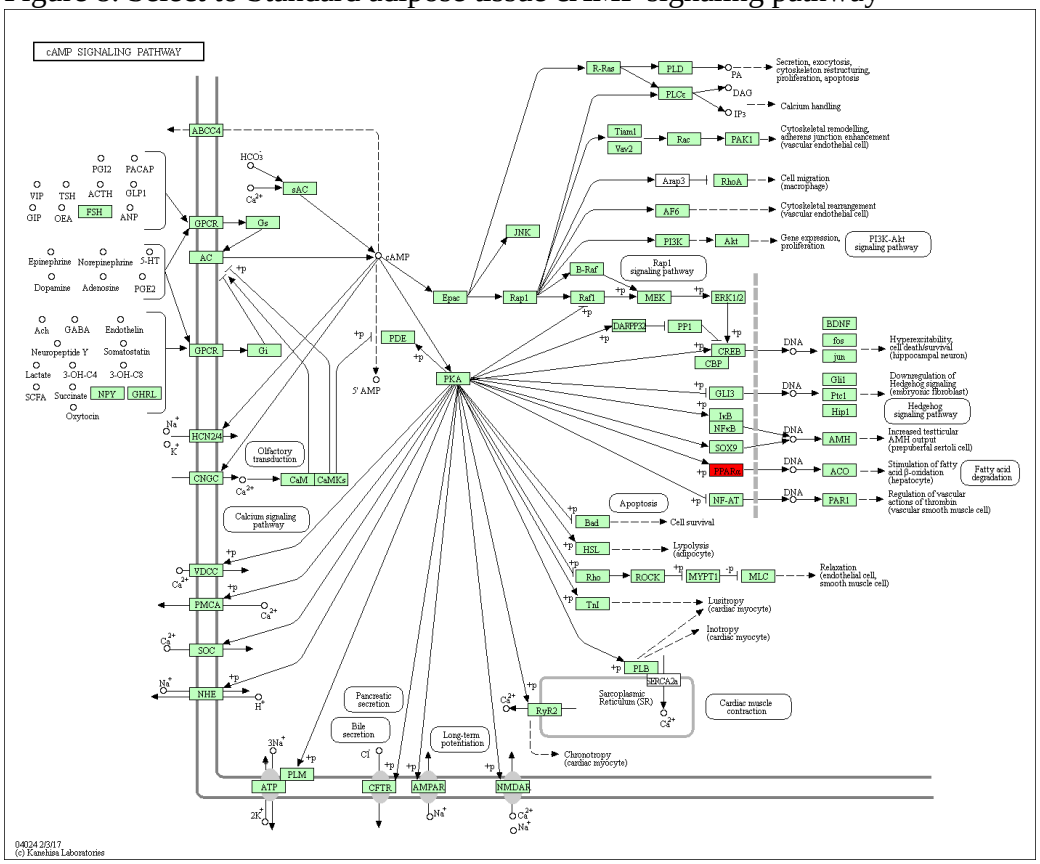


Figure 9. Select to Standard adipose tissue PPAR signaling pathway

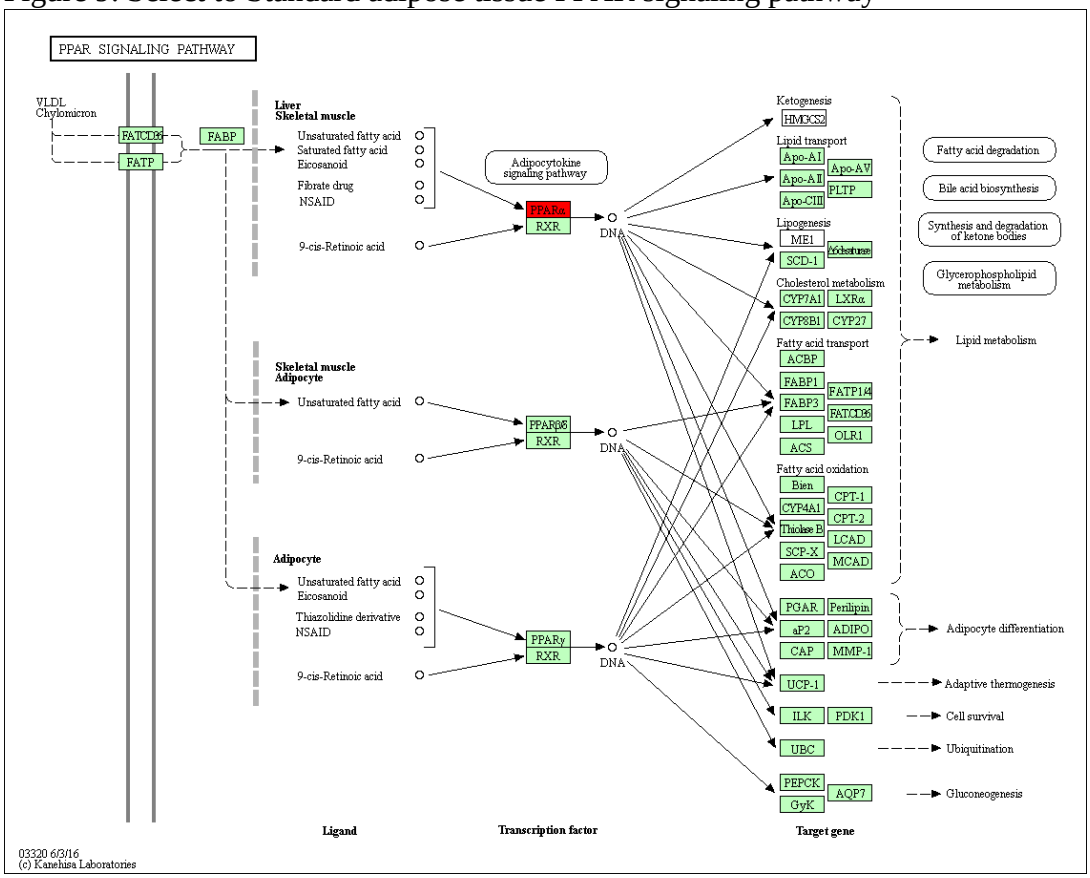


Figure 10. Select to Choice adipose tissue adipocytokine signaling pathway

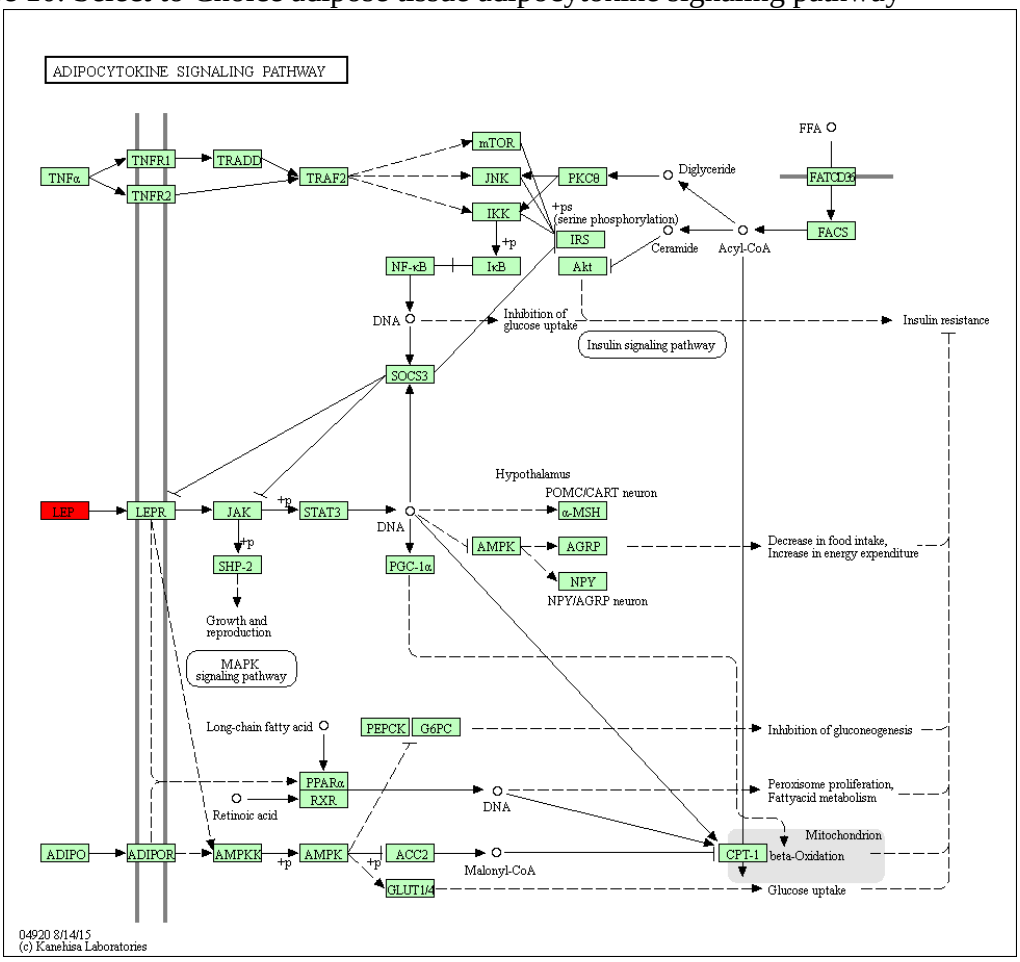


Figure 11. Select to Choice adipose tissue AMPK signaling pathway

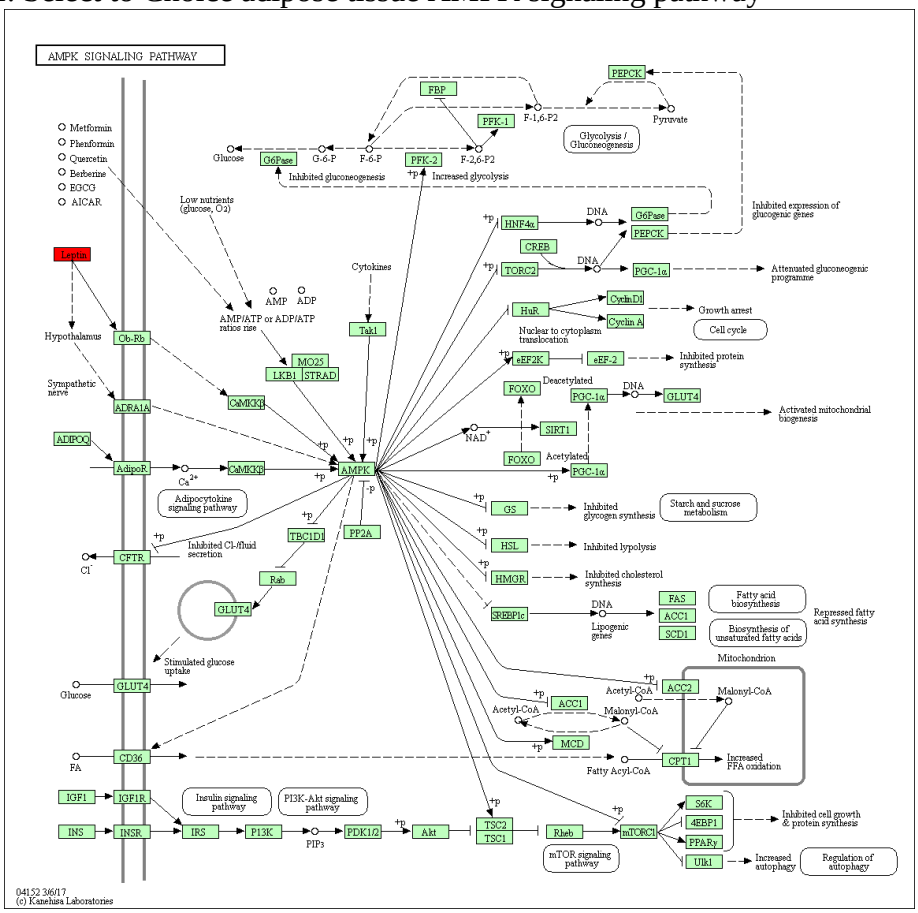


Figure 12. Select to Choice adipose tissue JAK-STAT signaling pathway

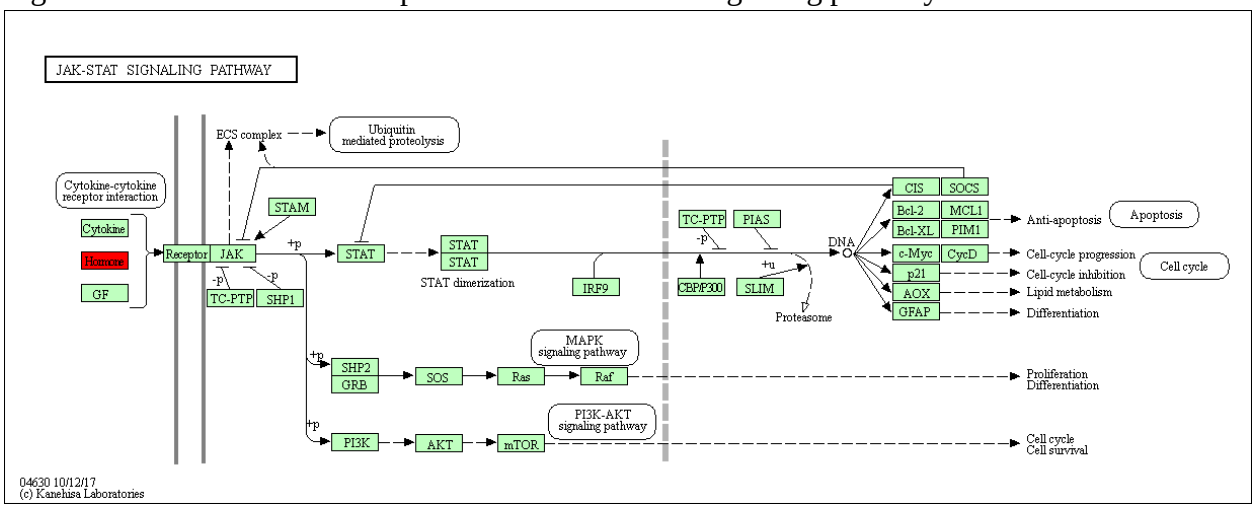


Figure 13. Select to Choice adipose tissue cAMP signaling pathway

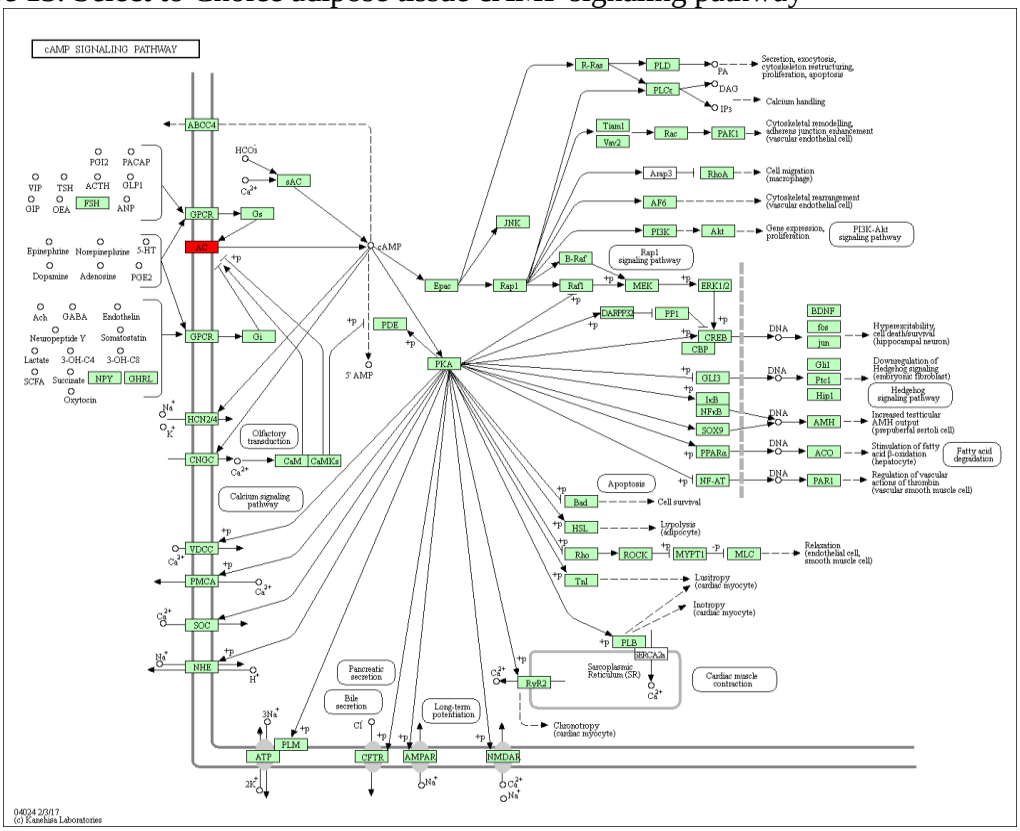


Figure 14. Select to Choice adipose tissue Rap1 pathway

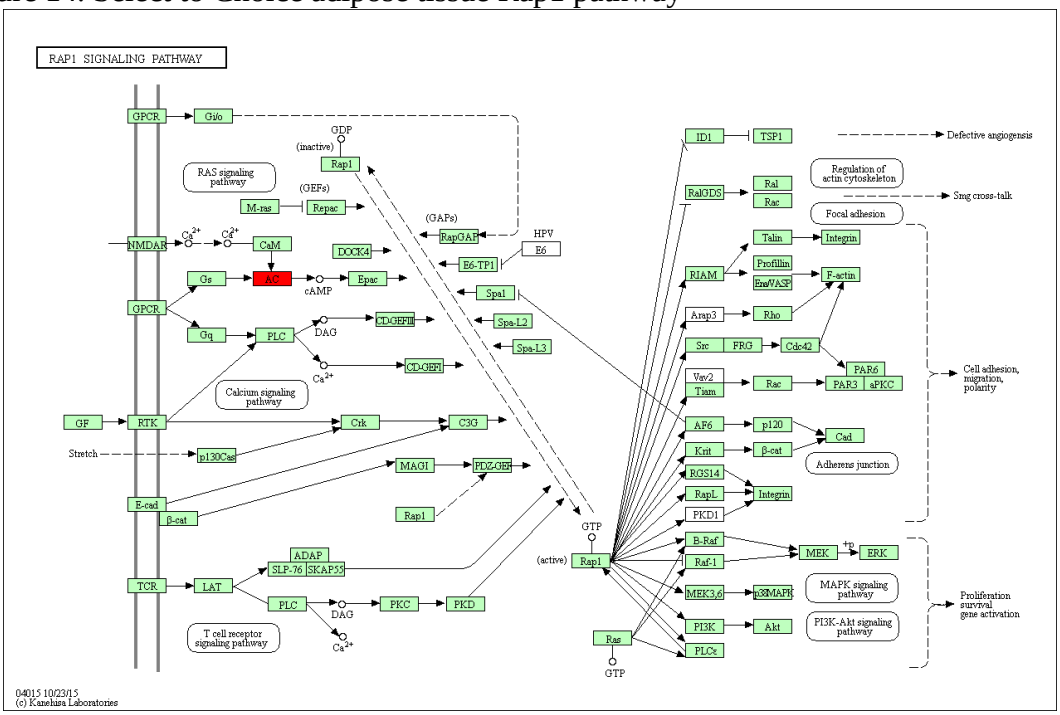


Figure 15. Choice to Standard adipose tissue adipocytokine signaling pathway

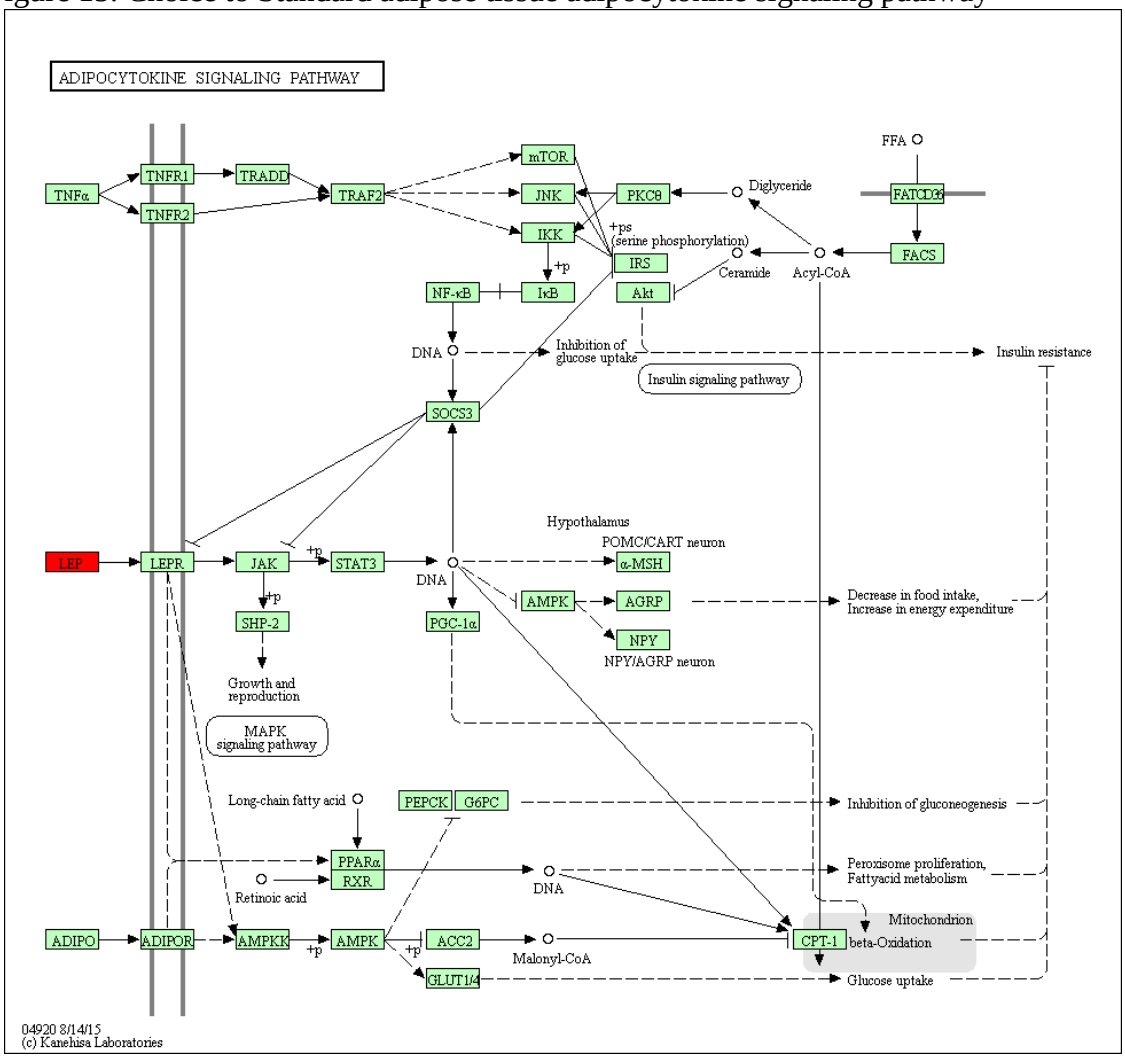


Figure 16. Choice to Standard adipose tissue AMPK signaling pathway

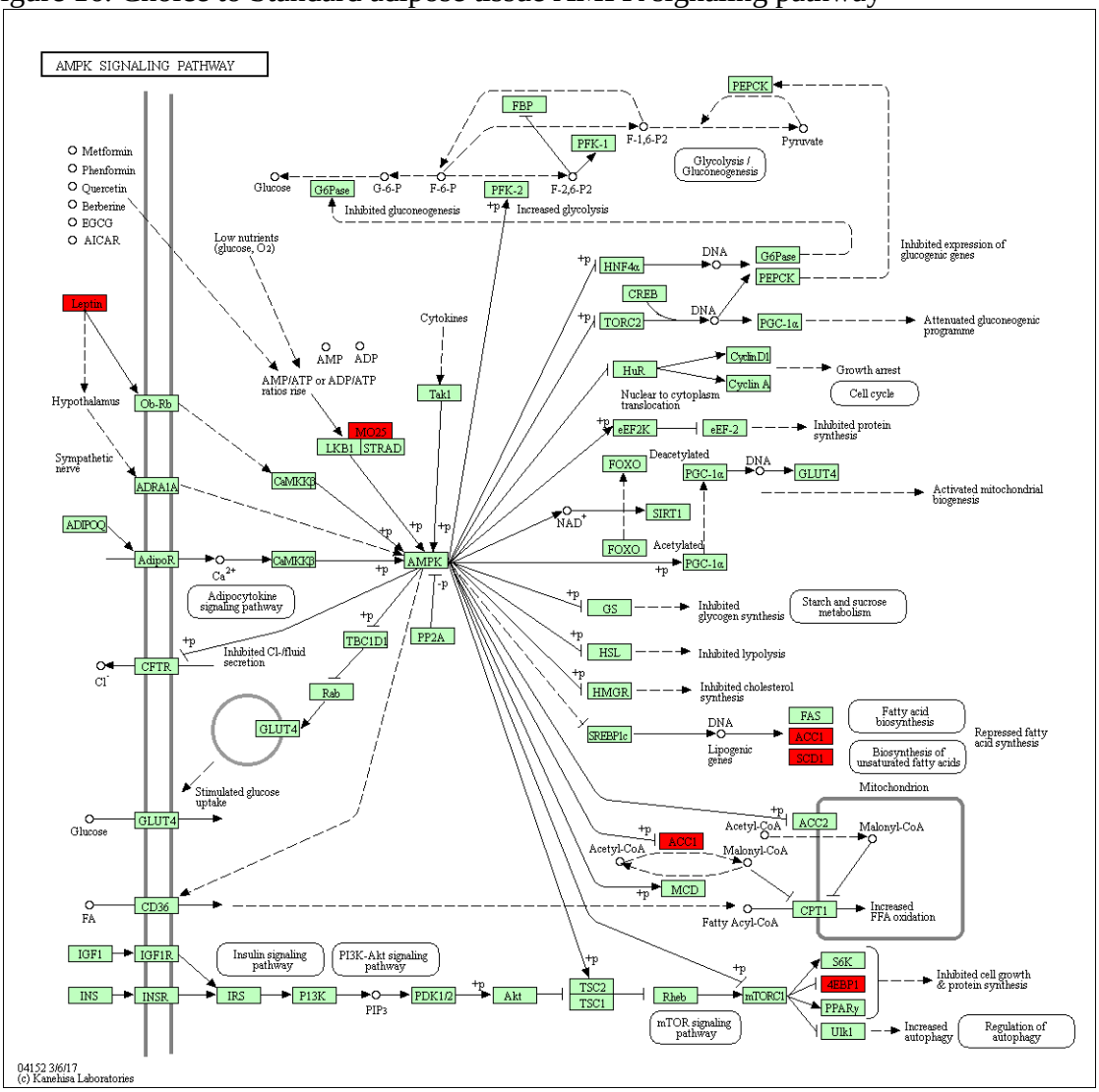


Figure 17. Choice to Standard adipose tissue cAMP signaling pathway

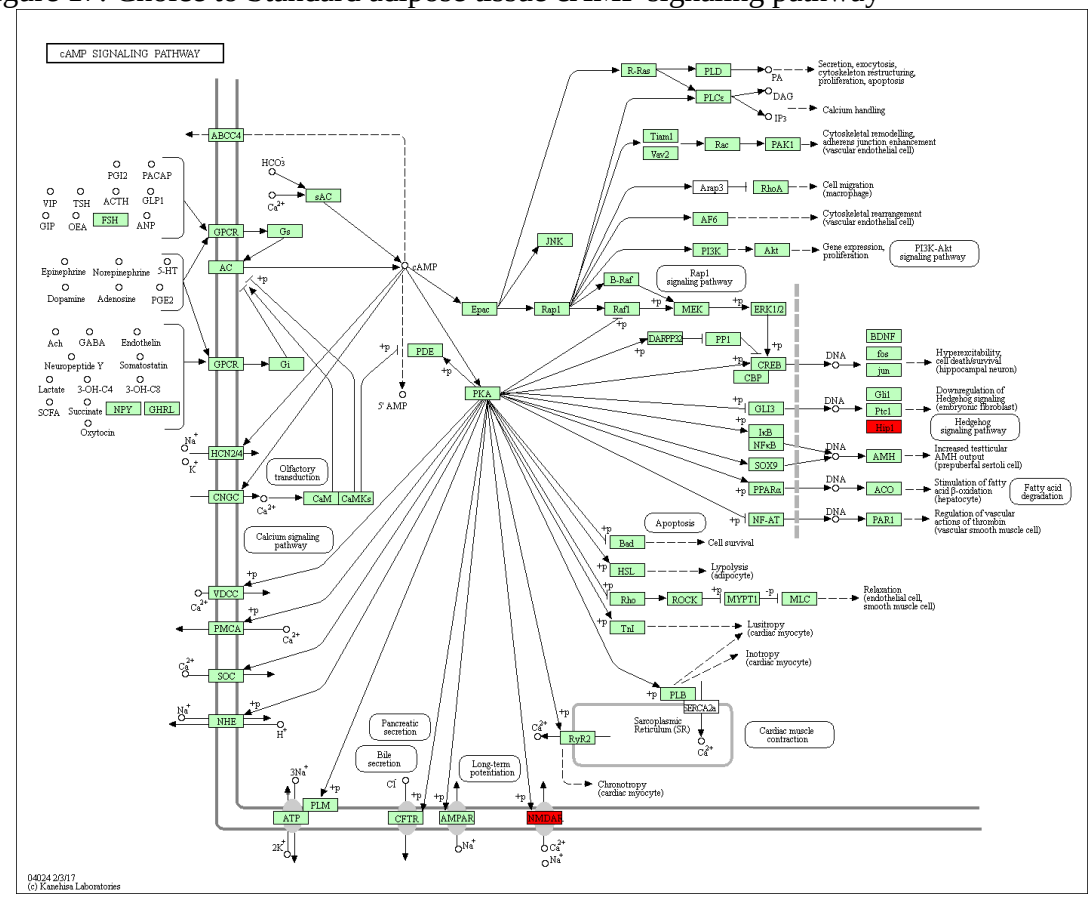


Figure 18. Choice to Standard adipose tissue fatty acid biosynthesis

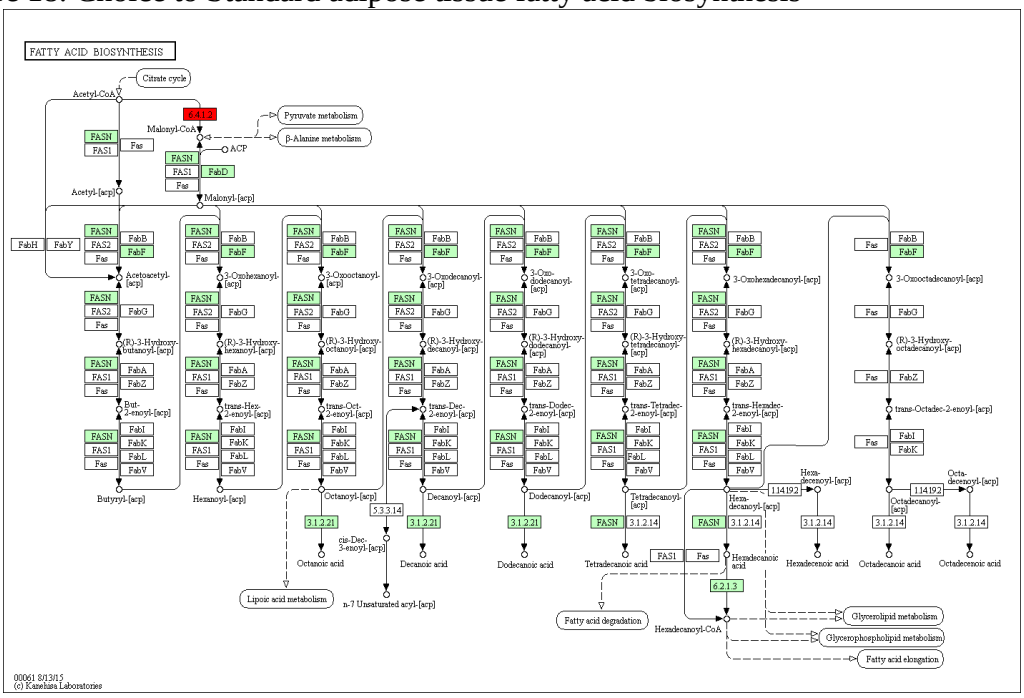


Figure 19. Choice to Standard adipose tissue fatty acid elongation

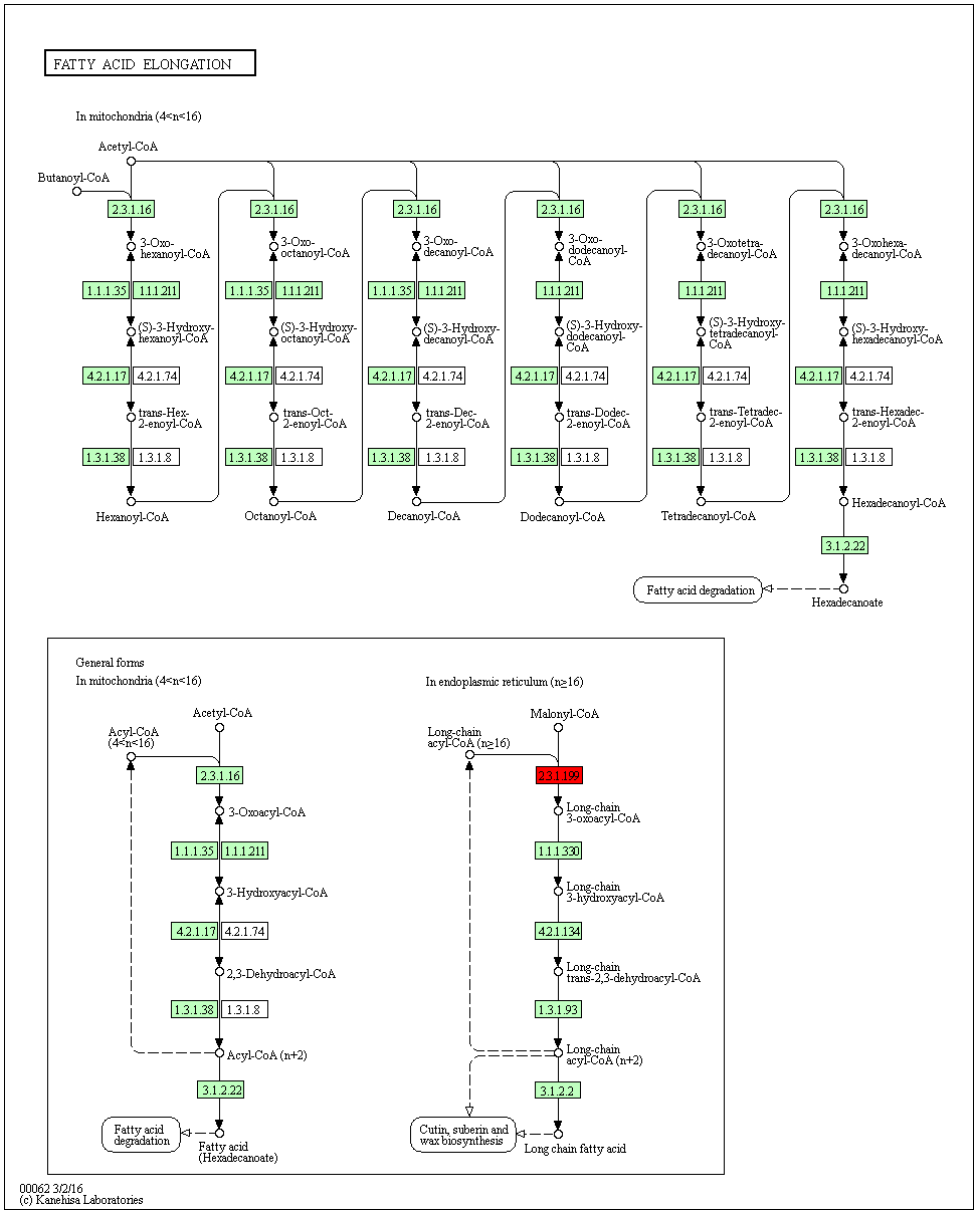


Figure 20. Choice to Standard adipose tissue glycolysis and gluconeogenesis pathway

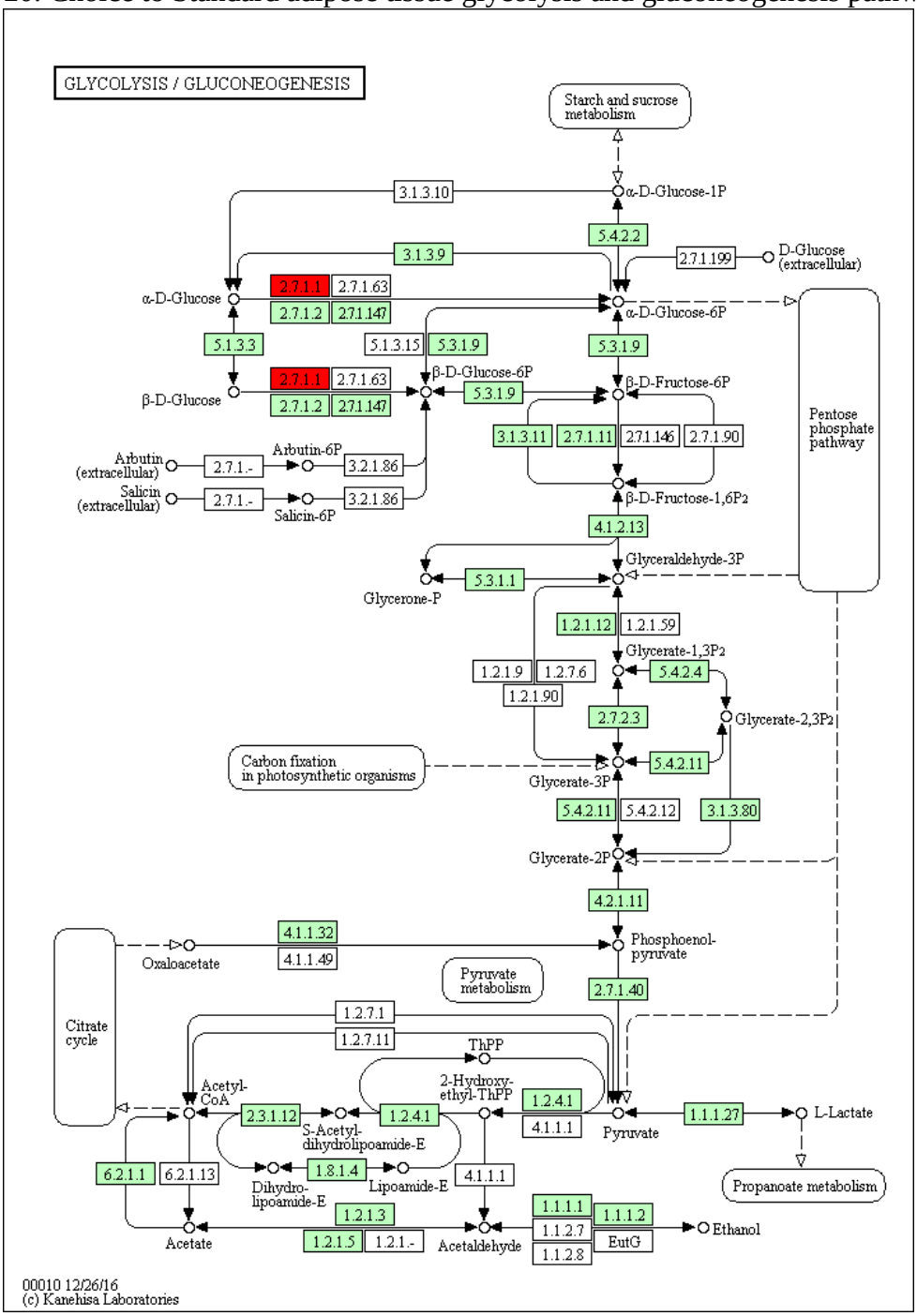


Figure 21. Choice to Standard adipose tissue insulin signaling pathway

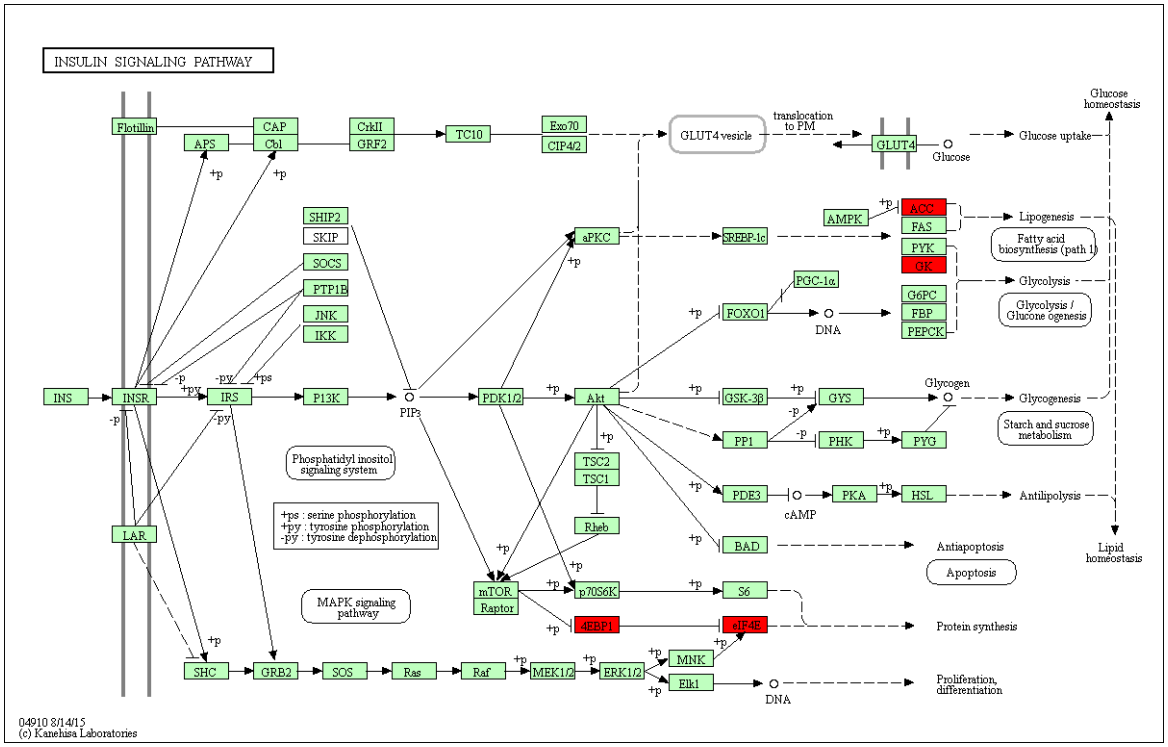


Figure 22. Choice to Standard adipose tissue JAK-STAT signaling pathway

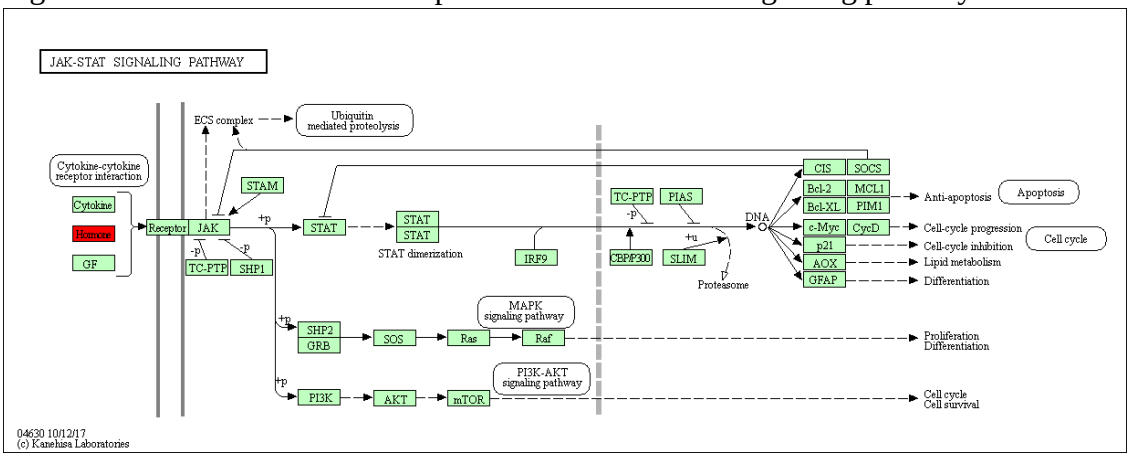


Figure 23. Choice to Standard adipose tissue PI3K-Akt signaling pathway

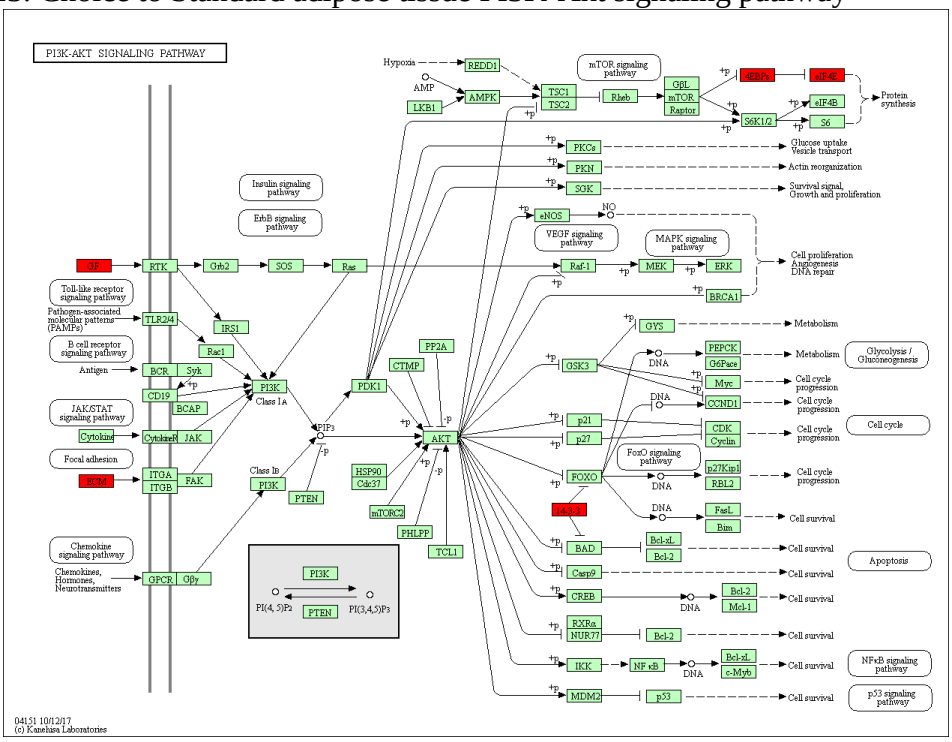


Figure 24. Choice to Standard adipose tissue PPAR signaling pathway

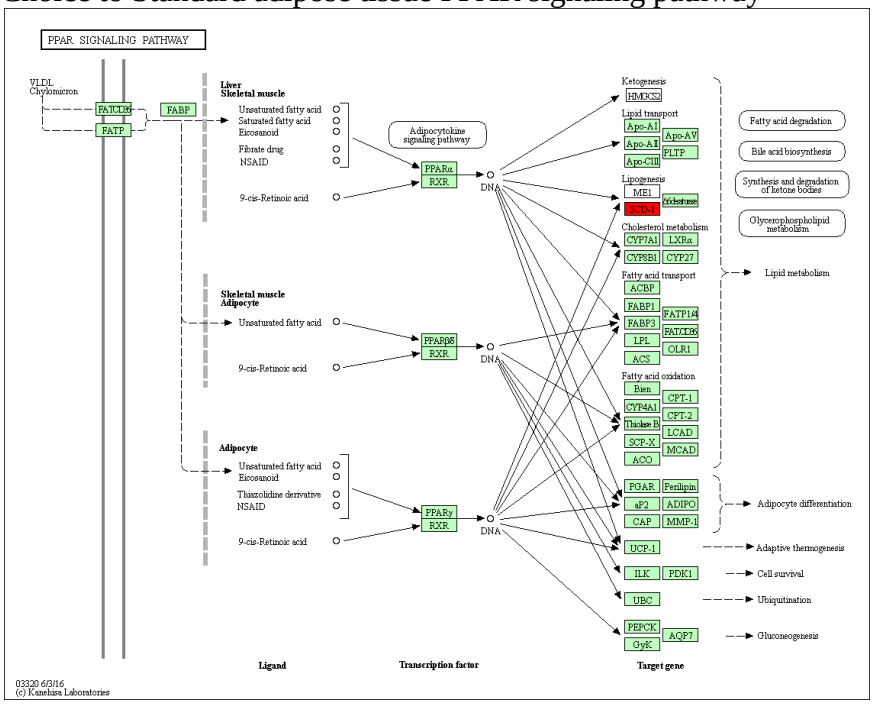


Figure 25. Choice to Standard adipose tissue Rap1 pathway

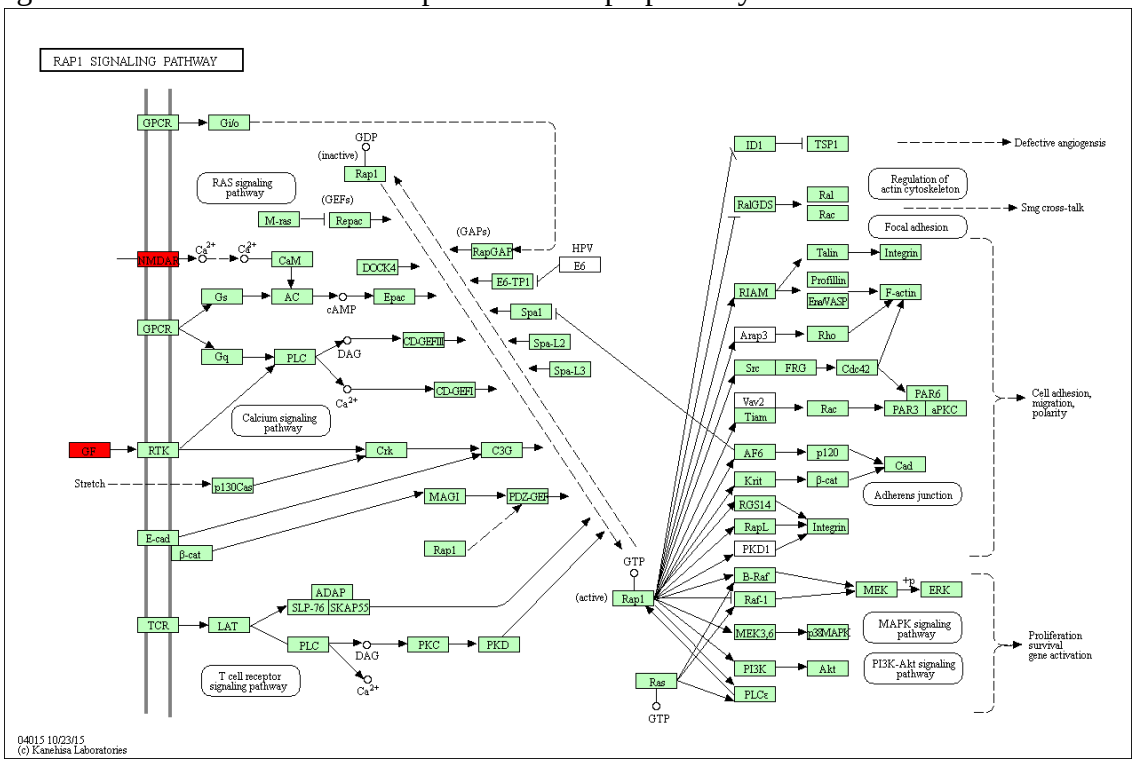


Figure 26. Standard to Choice muscle tissue AMPK signaling pathway

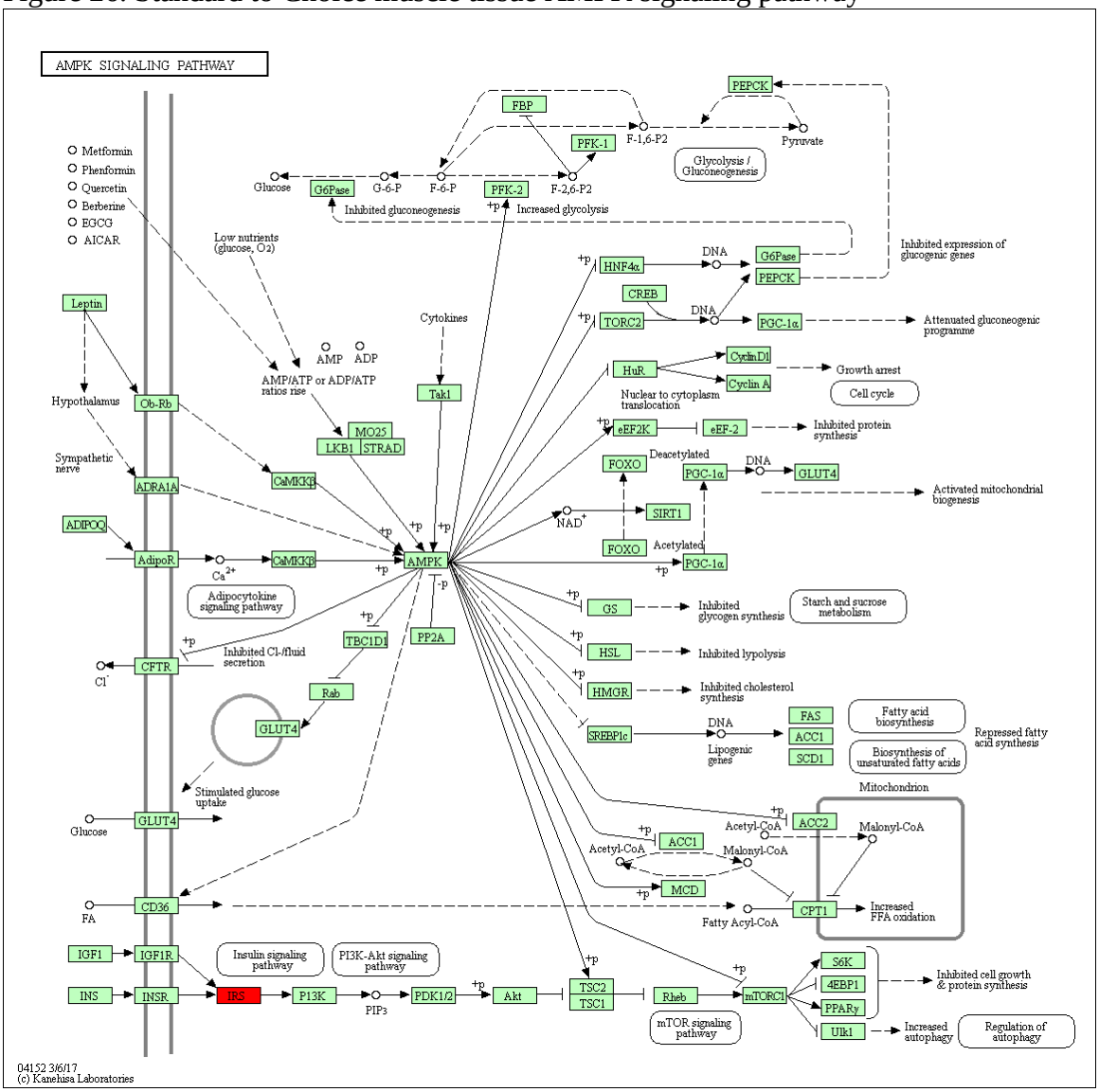


Figure 27. Standard to Choice muscle tissue FOXO signaling pathway

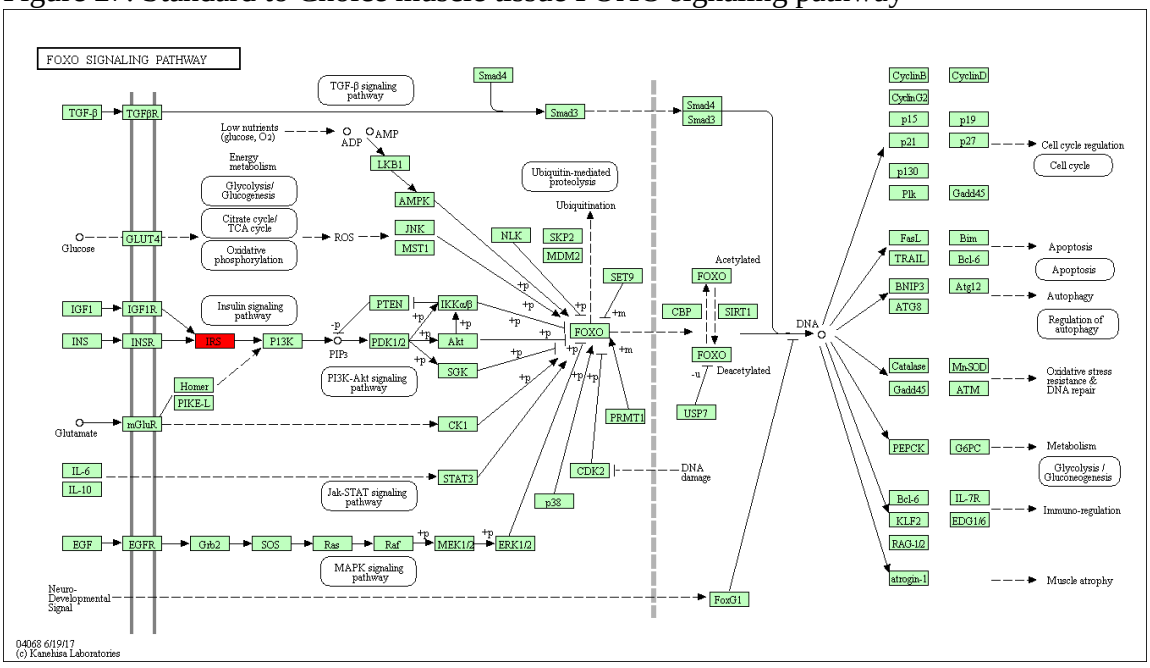


Figure 28. Standard to Choice muscle tissue mTOR signaling pathway

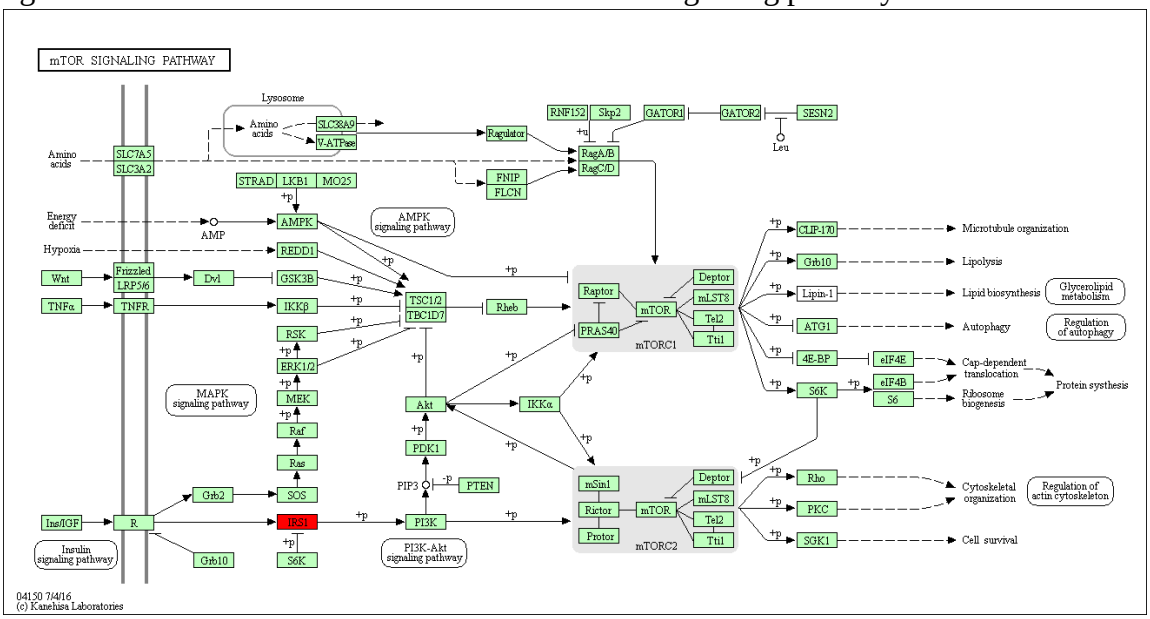


Figure 29. Standard to Choice muscle tissue PI3K-Akt signaling pathway

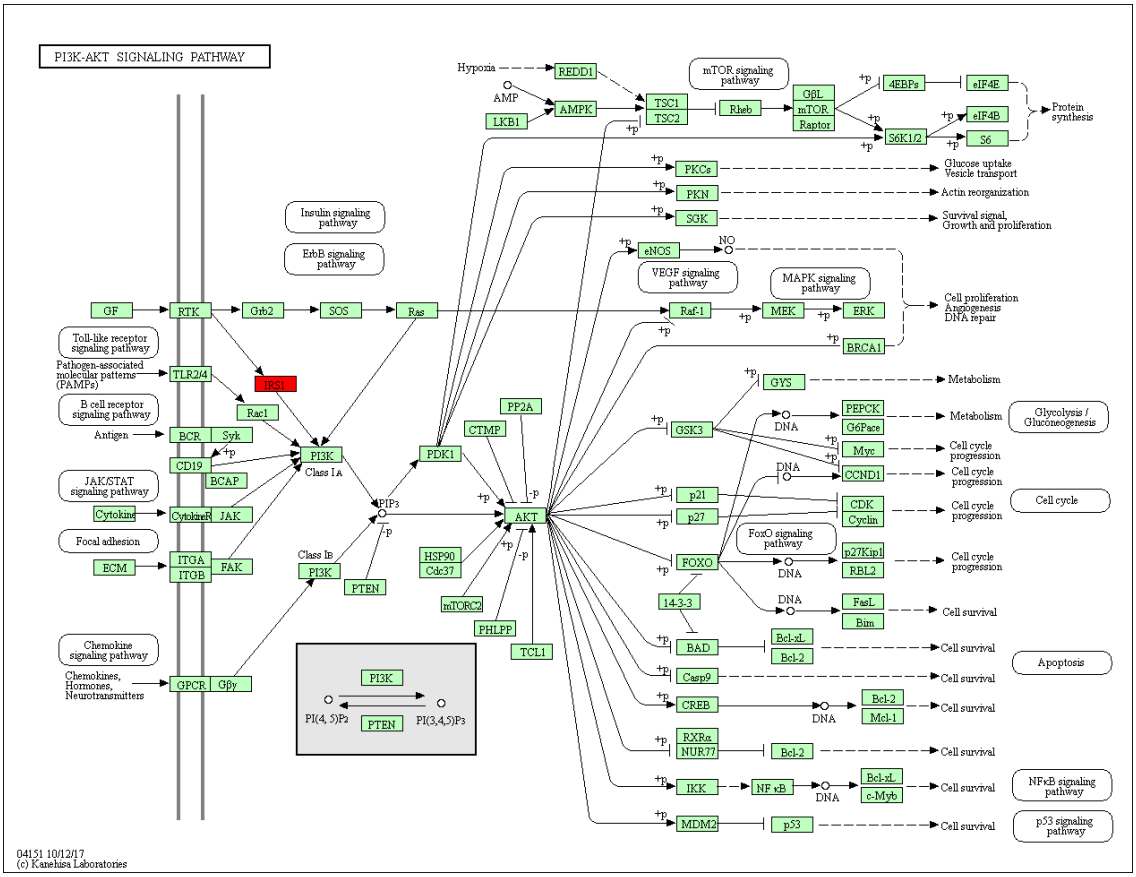


Figure 30. Asthma pathway

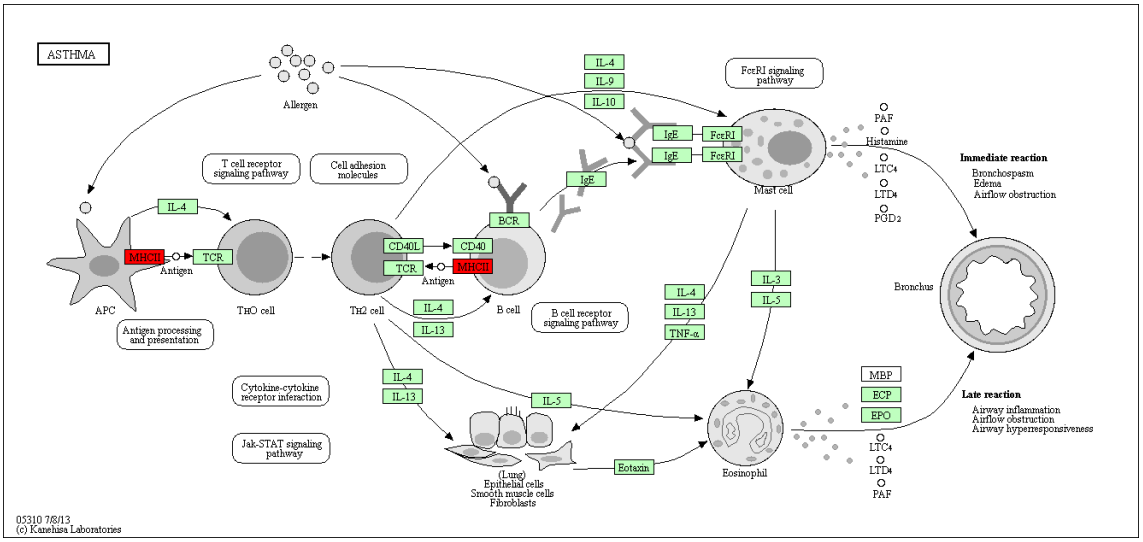
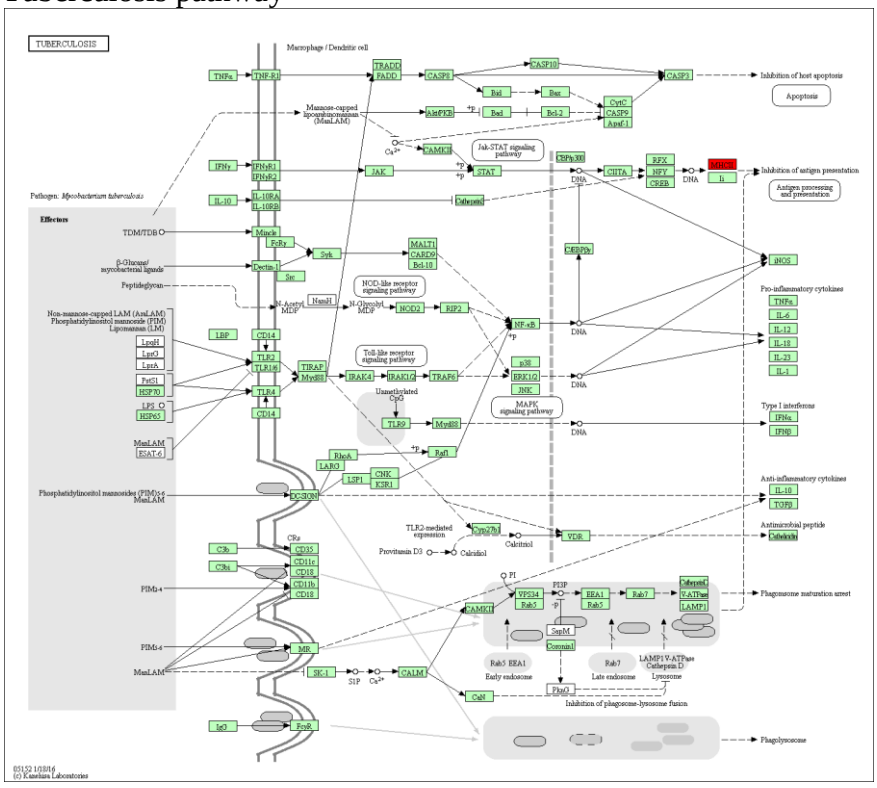


Figure 31. Tuberculosis pathway



As seen above, the comparison that showed the greatest alteration in the expression of genes and pathways occurred between the Choice to Standard adipose tissue. While there were differences between Select to Choice and Select to Standard, these differences seem to be related to the deposition and regulation of normal adipose tissue and did not seem relevant to deposition of intramuscular fat or marbling.

Of the differences between Choice to Standard adipose tissue, ones that could have a large impact are the inhibition of the mTOR signaling pathway, apoptosis, and the regulation of actin cytoskeleton. Of the pathways potentially promoted, lipid metabolism, apoptosis, actin reorganization could play a large part.

As adipose tissue is deposited intramuscularly, it displaces muscle tissue. This could potentially cause apoptosis, which is programmed cell death, in order to make room for the developing and depositing adipose tissue. This might promote the muscle fibers, even sarcomeres themselves, to weaken as the adipose tissue is deposited. There is also a potential of actin reorganization, which could also play a part in the weakening of muscle to allow for the deposit of adipose tissue.

With regards to Standard to Choice muscle tissue samples there was potential for the protein synthesis in Standard, while lipolysis had the possibility to be upregulated in the Standard compared to Choice. This would agree with what is often seen, less intramuscular adipose tissue deposited in Standard when compared to Choice, as the Standard animal is still developing muscle as well as other depots of adipose tissue, such as KPH, subcutaneous, and intermuscular. There is also a potential for the reorganization

of muscle as adipose tissue is being deposited, specifically with regards to actin reorganization, microtubule organization, and protein synthesis.

Protein-protein Interaction

Protein-protein interaction is when physical contacts of high specificity between two or more protein molecules as a result of biochemical events steered by electrostatic forces. In this study there was a total of three instances of this recorded, all occurring between the Choice and Standard adipose samples (table 21). In order to be considered having high specificity, the STRING score must be above 700, which in all three interactions it was. Due to a lack of information with regards to bovine protein-protein interaction, it is unsure at this time the impact they could have.

Table 21. Protein-protein interactions

Down regulation		
Protein 1	Protein 2	STRING score
ENSBTAP00000012530	ENSBTAP00000039615	964
ENSBTAP00000019800	ENSBTAG00000021077	800
Up regulation		
Protein 1	Protein 2	STRING score
ENSBTAG00000013108	ENSBTAG00000020127	800

Transcription Factor Analysis

Transcription factors are proteins that control the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence. Their function is to regulate genes in order to assure that they are expressed at the correct time, in the correct amount, throughout the life of the cell and the organism. There were a total

of 1,320 transcription factors that were expressed in the tissues evaluated in this study, comprised of 69 families (table 22).

Table 22. Transcription factors

Transcription family	Count
CBF	1
GCR	1
NF-YA	1
Nrf1	1
Oestrogen receptor	1
PC4	1
Progesterone receptor	1
zf-BED	1
CG-1	2
CSL	2
Ecdystd receptor	2
GCM	2
HMGI/HMGY	2
NDT80/PhoG	2
NF-YB/C	2
Nuclear orphan receptor	2
Prox1	2
zf-LITAF-like	2
zf-NF-X1	2
COUP	3
GTF2I	3
HTH	3
Other nuclear receptor	3
Others	3
P53	3
PPAR receptor	3
Runt	3
TF_Otx	3
TSC22	3
AF-4	4
CUT	4
ROR receptor	4
SRF	4
TEA	4
AP-2	5

Transcription family	Count
COE	5
Tub	5
SAND	6
zf-C2HC	6
CP2	7
DM	7
zf-MIZ	7
MBD	8
MH1	8
PAX	8
Retinoic acid receptor	8
STAT	8
RFX	9
CSD	10
IRF	10
RHD	10
E2F	11
THAP	11
ARID	13
CTF/NFI	14
Pou	14
T-box	15
zf-GATA	15
Thyroid hormone receptor	23
MYB	25
ETS	26
HSF	34
Fork head	37
ZBTB	41
TF_bZIP	42
HMG	47
bHLH	95
Homeobox	176
zf-C2H2	474
Total	1,320

CONCLUSION

The expected gene expression changes related to muscle growth, fat deposition, fatty acid synthesis and regulation were observed in this study. However, organisms are complex systems, with metabolic processes under multiple levels of regulation. This can cause some of the conflicting results that were seen with genes being regulated to opposite effects. Overall there was evidence of many genes being differentially expressed, particularly between the Choice and Standard adipose tissue. There were few genes being differentially expressed between muscle tissue groups. This can potentially be interpreted to demonstrate that the adipose tissue and surrounding muscle can play a bigger role in meat quality and tenderness levels than currently known, and that the muscle tissue itself plays a minor role.

This study emphasized many genes and pathways related to adipose tissue in particular that need to be investigated further. In particular, the pathways of fatty acid biosynthesis pathway; PI3-Akt signaling pathway due to its impact on apoptosis and actin reorganization,; AMPK signaling pathway because of its potential impact on fatty acid biosynthesis; and JAK-STAT signaling pathway due to its impact on apoptosis, lipid metabolism and the PI3K-Akt signaling pathway. The pathways related to muscle tissue needing additional research are the mTOR signaling pathway due to its impact on microtubule organization, protein synthesis and the regulation of actin cytoskeleton, as well as the PI3K-Akt signaling pathway due to its impact on apoptosis, protein synthesis and actin reorganization.

Genes of particular importance that also need further research with regards to adipose tissue are leptin, due to its impact on both the JAK-STAT and AMPK signaling pathway; CAB39L, ACC1, SCD1 and EIF4EBP1 due to their impact on the AMPK signaling pathway; and NGF, RELN, EIF4EBP1, EIF4E, YWHAG and FGF1 because of their impact on the PI3K-Akt signaling pathway. The muscle-related gene needing additional research is IRS1, which influenced both the mTOR and PI3K-Akt signaling pathways.

It does appear from this study that the role of adipose tissue plays in meat quality and tenderness may provide more potential selection targets and variables that can be used to improve meat quality than muscle tissue. This is logical as at these points, muscle is already fairly developed and the body is transitioning to the deposition of adipose instead.

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APPENDICES

APPENDIX A

DIFFERENTIALLY EXPRESSED GENES

Table 1. All Select to Standard adipose tissue differentially expressed genes

Gene code	Select read count	Standard read count	P-value	Gene abbreviation	Gene name
ENSBTAG00000004005	267.2275588	116.6258825	4.02E-05	WDR7	WD repeat domain 7
ENSBTAG00000004899	1190.30506	470.4327028	6.44E-05	ABLIM1	actin binding LIM protein 1
ENSBTAG00000004966	906.3176645	377.1467654	3.56E-05	KIF1B	kinesin family member 1B
ENSBTAG00000006037	1007.415135	285.9912824	3.03E-05	WISP2	WNT1 inducible signaling pathway protein 2
ENSBTAG00000008063	466.1987041	193.6748103	2.47E-05	PPARA	peroxisome proliferator activated receptor alpha
ENSBTAG00000008083	718.7380485	246.0446055	6.21E-05	SEL1L	protein sel-1 homolog 1 precursor
ENSBTAG00000009014	0.799209984	13.34036158	6.01E-07	UPK1B	uroplakin 1B
ENSBTAG00000009267	265.3419679	101.4785113	5.73E-05	UHRF1BP1	UHRF1 binding protein 1
ENSBTAG00000012558	320.2979088	115.1218927	7.06E-06	ADAMTS12	ADAM metallopeptidase with thrombospondin type 1 motif 12
ENSBTAG00000013236	431.2773864	209.0811984	2.73E-05	MED1	mediator complex subunit 1
ENSBTAG00000013802	129.6054072	46.48940799	6.59E-06	DAB1	DAB1, reelin adaptor protein
ENSBTAG00000014367	138.9019305	58.36935619	4.78E-06	PRKX	protein kinase, X-linked
ENSBTAG00000014387	52.33053568	13.48899581	2.19E-05	PRKAB2	protein kinase AMP-activated non-catalytic subunit beta 2
ENSBTAG00000016757	136.800362	31.56619105	1.70E-05	ZKSCAN1	zinc finger with KRAB and SCAN domains 1
ENSBTAG00000017761	616.0282848	200.1258414	1.51E-05	SMC1A	structural maintenance of chromosomes protein 1A
ENSBTAG00000018744	123.8207662	40.42256951	6.89E-07	MGAT5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase
ENSBTAG00000019954	110.0636364	47.62546524	6.32E-05	ABHD2	abhydrolase domain containing 2
ENSBTAG00000020799	241.0480965	109.9843797	2.62E-05	EXOC6B	exocyst complex component 6B
ENSBTAG00000021077	0	446.4814534	3.70E-08	-//-	

ENSBTAG00000025964	743.1964189	316.8595318	4.53E-05	MAST4	microtubule associated serine/threonine kinase family member 4
ENSBTAG00000027642	83.98793843	20.03062614	4.54E-05	C11orf42	chromosome 11 open reading frame 42
ENSBTAG00000038442	41.96577289	13.3960317	8.21E-06	WDR87	WD repeat domain 87
ENSBTAG00000040128	3605.795337	1527.681135	5.96E-05	FZD4	frizzled class receptor 4
ENSBTAG00000040427	57.84772767	22.25602976	6.20E-05	-//-	
ENSBTAG00000046509	107.7653773	40.44647977	9.01E-06	FAM46C	family with sequence similarity 46 member C
ENSBTAG00000047547	4.27220393	463.9217472	5.18E-43	-//-	
Novel00336	20.28142458	56.98787402	6.01E-05	-//-	
Novel01055	29.66890521	9.149998868	6.36E-05	--	PREDICTED: LOW QUALITY PROTEIN: zinc finger protein 551 [Bos taurus]
Novel01274	25.73052676	6.931650805	5.20E-05	--	PREDICTED: peroxisomal N(1)-acetyl-spermine/spermidine oxidase isoform X4 [Ovis aries musimon]
Novel01930	522.1183631	229.8450246	9.67E-06	-//-	
Novel02594	153.9037494	69.47577876	3.36E-05	--	hypothetical protein, conserved [Babesia bigemina]
Novel02679	20.43745361	5.018698953	5.51E-05	--	PREDICTED: lysine-specific demethylase 4C-like [Tursiops truncatus]
Novel02852	40.32871393	14.9566925	6.32E-05	--	PREDICTED: transmembrane 9 superfamily member 2-like isoform X2 [Bos taurus]

Table 2. All Select to Choice adipose tissue differentially expressed genes

Gene code	Select reads	Choice reads	P-value	Gene abbreviation	Gene name
ENSBTAG00000000246	93.57279	242.8505	4.72E-06	ME3	malic enzyme 3
ENSBTAG00000002046	1622.573	747.691	3.29E-05	-//-	
ENSBTAG00000004836	35.63447	11.64995	3.63E-05	-	nik-related protein kinase
ENSBTAG00000007860	54.59434	19.44777	1.50E-05	ASPM	abnormal spindle microtubule assembly
ENSBTAG00000010682	2522.631	8199.678	3.34E-07	DDR1	epithelial discoidin domain-containing receptor 1 precursor
ENSBTAG00000010774	72.09664	24.89427	5.29E-06	NUSAP1	nucleolar and spindle associated protein 1
ENSBTAG00000012638	73.03628	190.5465	5.60E-06	S100A12	S100 calcium binding protein A12
ENSBTAG00000013107	103.7719	281.0858	1.10E-05	SHANK1	SH3 and multiple ankyrin repeat domains 1
ENSBTAG00000014340	914.4057	322.5862	1.60E-06	KERA	keratocan
ENSBTAG00000014911	2371.164	8660.136	2.46E-05	LEP	leptin
ENSBTAG00000015606	776.855	1673.526	3.64E-05	KAZN	kazrin, periplakin interacting protein
ENSBTAG00000018777	818.903	2158.209	6.08E-07	ADCY5	adenylate cyclase type 5
ENSBTAG00000019262	164.7217	55.94236	9.34E-06	TOP2A	topoisomerase (DNA) II alpha
ENSBTAG00000020510	7.363604	33.47247	2.69E-05	EPHA10	EPH receptor A10
ENSBTAG00000024449	100.5042	36.31176	1.13E-05	CENPF	centromere protein F
ENSBTAG00000025071	98.78828	40.90729	3.44E-05	TENM2	teneurin transmembrane protein 2
ENSBTAG00000047569	45.98635	11.67487	2.52E-06	-//-	
Novel00469	63.13649	18.90977	2.17E-05	--	PREDICTED: taste receptor type 2 member 42-like, partial [Bos mutus]
Novel00632	190.0347	86.7887	2.80E-05	--	PREDICTED: carbonyl reductase [NADPH] 1-like isoform X2 [Ovis aries musimon]

Novel00720	85.55824	28.22112	1.55E-05	--	PREDICTED: extracellular calcium-sensing receptor-like [Equus caballus]
Novel01757	266.9865	58.33762	4.92E-09	--	PREDICTED: EF-hand calcium-binding domain-containing protein 1 isoform X5 [Ovis aries musimon]
Novel02471	90.95808	25.75499	4.06E-08	--	hypothetical protein, conserved [Babesia bigemina]
Novel03001	56.70565	20.14489	2.77E-05	--	PREDICTED: protein FAM209A-like [Capra hircus]

Table 3. All Choice to Standard adipose tissue differentially expressed genes

Gene code	Choice reads	Standard reads	P-value	Gene abbreviation	Gene name
ENSBTAG00000000246	250.7052991	73.68379006	1.89E-09	ME3	malic enzyme 3
ENSBTAG00000000434	7784.499258	3431.10319	0.00012813	CRYAB	Alpha-crystallin B chain
ENSBTAG00000000590	226.9117871	86.6078087	6.78E-06	POLE	DNA polymerase epsilon catalytic subunit A
ENSBTAG00000000795	390.8553402	205.3570194	0.00023351	NMNAT2	nicotinamide nucleotide adenylyltransferase 2
ENSBTAG00000001165	1162.853956	2374.914417	0.00032838	AIFM2	Bos taurus apoptosis-inducing factor, mitochondrion-associated, 2 (AIFM2), mRNA. mRNA;Acc:NM_001314031]
ENSBTAG00000001639	64.58725422	19.94262221	0.00017681	TRPM1	transient receptor potential cation channel subfamily M member 1
ENSBTAG00000002046	770.8317745	2351.932618	9.89E-05	-//-	
ENSBTAG00000002129	45.907215	168.8853594	4.22E-05	KLF5	Kruppel like factor 5
ENSBTAG00000002356	403.832636	64.55735831	8.60E-08	RBM47	RNA binding motif protein 47

ENSBTAG00000002915	99.52090129	20.93112191	3.52E-05	GPR63	G protein-coupled receptor 63
ENSBTAG00000003113	361.6009376	82.66910702	7.97E-06	RNF125	ring finger protein 125
ENSBTAG00000003192	1984.509382	896.6898214	1.85E-05	TBC1D16	TBC1 domain family member 16
ENSBTAG00000003196	579.101913	256.863223	0.0001318	PAPSS2	bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2
ENSBTAG00000003359	18780.52553	6929.674559	4.39E-06	ELOVL5	ELOVL fatty acid elongase 5
ENSBTAG00000003650	142.1944924	291.1420581	0.00025272	NR4A2	nuclear receptor subfamily 4 group A member 2
ENSBTAG00000003658	70.73350439	166.1783114	4.50E-05	RELN	reelin precursor
ENSBTAG00000004005	261.4677221	125.5170888	0.00030617	WDR7	WD repeat domain 7
ENSBTAG00000004034	504.963705	146.939931	0.00018661	SESN3	sestrin 3
ENSBTAG00000004077	8903.965131	4647.780626	0.00032637	YWHAG	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma Symbol;Acc:HGNC:12852]
ENSBTAG00000004204	510.303486	213.0706758	7.43E-05	GPR107	protein GPR107
ENSBTAG00000004300	84.50046528	34.40160517	0.00022617	LRRC8E	leucine rich repeat containing 8 family member E
ENSBTAG00000004720	1140.145198	587.6405401	0.00019042	SULF1	sulfatase 1
ENSBTAG00000005198	506.3863588	145.3035765	2.62E-07	FGF1	Fibroblast growth factor 1
ENSBTAG00000005586	417.1646124	1039.848698	0.00015378	GATM	glycine amidinotransferase
ENSBTAG00000005882	227.5664387	108.572303	0.00022702	ZNF333	zinc finger protein 333
ENSBTAG00000006673	14.60536802	3.186135254	0.00013682	TMEM179	transmembrane protein 179
ENSBTAG00000007100	720.9799428	317.5857976	0.00017407	TRAPPC10	trafficking protein particle complex subunit 10
ENSBTAG00000007273	4331.143363	660.5063872	1.84E-05	TF	transferrin
ENSBTAG00000007348	3.924234092	22.8124883	0.00011414	STRA6	stimulated by retinoic acid 6
ENSBTAG00000007446	25.43454316	71.64164715	8.45E-05	NGF	nerve growth factor
ENSBTAG00000007554	286.7050558	687.7824501	7.10E-05	IFI6	interferon alpha inducible protein 6
ENSBTAG00000007883	20.87941763	64.52713453	3.80E-06	-//-	

ENSBTAG00000007962	2508.336262	950.7036823	1.08E-05	ATP9A	ATPase phospholipid transporting 9A (putative)
ENSBTAG00000008083	770.9016596	264.6692662	0.00027007	SEL1L	protein sel-1 homolog 1 precursor
ENSBTAG00000008102	449.5864342	88.51469632	1.47E-10	CRTAC1	cartilage acidic protein 1 isoform 2 precursor
ENSBTAG00000008153	1208.256742	592.0753436	0.00015581	CAMSAP2	calmodulin regulated spectrin associated protein family member 2
ENSBTAG00000008718	22.09564616	119.4963014	0.0003164	GALNT17	polypeptide N-acetylgalactosaminyltransferase 17
ENSBTAG00000008731	3936.465232	1787.835603	7.13E-05	PRDX3	peroxiredoxin 3
ENSBTAG00000008793	115.5175062	318.0525096	9.59E-05	-	Ribonuclease pancreatic
ENSBTAG00000008839	104.2225857	43.1304875	0.00030046	AMER1	APC membrane recruitment protein 1
ENSBTAG00000008953	839.7005392	1724.658702	0.00015085	TAP1	transporter 1, ATP binding cassette subfamily B member
ENSBTAG00000009020	1588.852392	795.759823	0.00011775	CRIM1	cysteine rich transmembrane BMP regulator 1
ENSBTAG00000009124	60.94627799	196.8139265	0.0001342	FEZ1	fasciculation and elongation protein zeta 1
ENSBTAG00000009656	45.94515343	133.4541166	5.51E-06	-//-	
ENSBTAG00000010129	6.85947537	27.0226973	0.00027094	SLITRK5	SLIT and NTRK like family member 5
ENSBTAG00000010245	38.66500396	7.990488784	6.04E-05	SPRY3	sprouty RTK signaling antagonist 3
ENSBTAG00000010371	132.4182819	20.52099345	1.00E-05	CHAC1	ChaC glutathione specific gamma-glutamylcyclotransferase 1
ENSBTAG00000010457	307.3723061	144.8618899	0.00027081	NUAK1	NUAK family kinase 1
ENSBTAG00000010507	17.70476936	48.03666074	0.00019058	SLC22A16	solute carrier family 22 member 16
ENSBTAG00000010522	154.8755121	449.4070021	3.72E-06	SCART1	scavenger receptor family member expressed on T-cells 1
ENSBTAG00000010682	8465.071608	3207.930816	4.65E-07	DDR1	epithelial discoidin domain-containing receptor 1 precursor

ENSBTAG00000010719	604.9856401	1380.066199	0.00026176	ANGPTL1	angiopoietin like 1
ENSBTAG00000010793	42311.01119	22217.85077	0.00018533	CCDC80	coiled-coil domain containing 80
ENSBTAG00000010913	985.9225344	175.8777303	6.39E-06	SRXN1	sulfiredoxin 1
ENSBTAG00000011056	478.924219	168.1632382	3.52E-05	IDS	iduronate 2-sulfatase precursor
ENSBTAG00000011337	361.9663297	109.841627	3.38E-06	ANKRD33B	ankyrin repeat domain 33B
ENSBTAG00000011490	48.00169943	12.45635203	0.0001305	TLCD2	TLC domain containing 2
ENSBTAG00000012164	305.387429	71.06761959	2.63E-05	CP	ceruloplasmin precursor
ENSBTAG00000012307	54.53535778	137.1799179	1.69E-05	DTNA	dystrobrevin alpha
ENSBTAG00000012626	645.456104	274.8901731	1.45E-05	B4GALT5	beta-1,4-galactosyltransferase 5
ENSBTAG00000012991	813.1968235	277.6099451	0.00016775	PRUNE2	prune homolog 2
ENSBTAG00000013090	6.211781881	50.22041473	1.32E-05	NODAL	nodal growth differentiation factor
ENSBTAG00000013107	290.0689623	66.51991687	5.95E-10	SHANK1	SH3 and multiple ankyrin repeat domains 1
ENSBTAG00000013108	746.2792459	163.4152755	9.91E-05	HK2	hexokinase 2
ENSBTAG00000013899	1611.001045	741.2207175	3.86E-05	LARP4B	La ribonucleoprotein domain family member 4B
ENSBTAG00000013919	2226.193097	5980.916793	1.23E-06	-//-	
ENSBTAG00000014005	6906.8681	3350.216416	0.00023478	PPIP5K1	diphosphoinositol pentakisphosphate kinase 1
ENSBTAG00000014367	140.2823913	62.80375718	0.00021687	PRKX	protein kinase, X-linked
ENSBTAG00000014422	2111.332999	1013.460598	0.00021424	MTMR10	myotubularin related protein 10
ENSBTAG00000014529	26.26729629	68.49631413	4.98E-05	-//-	
ENSBTAG00000014536	558.6575698	198.4927234	2.51E-05	SFXN1	sideroflexin 1
ENSBTAG00000014615	107.2811422	17.79968995	2.12E-06	SLC26A2	solute carrier family 26 member 2
ENSBTAG00000014889	375.4091982	164.7915838	0.00015132	DCBLD2	discoidin, CUB and LCCL domain-containing protein 2
ENSBTAG00000014911	8938.900072	3504.577478	0.00029652	LEP	leptin
ENSBTAG00000015043	8114.583989	20064.49132	0.00033064	MFNG	MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase

ENSBTAG00000015177	4671.933098	1648.160975	1.09E-05	PRSS23	protease, serine 23
ENSBTAG00000015182	11.30698712	33.80167158	0.00017709	STARD10	StAR related lipid transfer domain containing 10
ENSBTAG00000015307	15.95464182	60.20747405	9.17E-05	FBN2	fibrillin 2
ENSBTAG00000015351	39.45406143	8.093726686	2.79E-05	STYK1	Bos taurus serine/threonine/tyrosine kinase 1 (STYK1), mRNA
ENSBTAG00000015419	1827.790243	687.3639809	1.85E-05	ARHGEF37	Rho guanine nucleotide exchange factor 37
ENSBTAG00000015536	876.5298816	320.6710168	7.94E-05	CPNE3	copine 3
ENSBTAG00000015571	622.8985834	267.8919456	0.00030916	GCLC	glutamate-cysteine ligase catalytic subunit
ENSBTAG00000015606	1728.286076	535.9301528	2.23E-09	KAZN	kazrin, periplakin interacting protein
ENSBTAG00000015607	452.8107606	143.9460943	1.32E-05	ERMP1	endoplasmic reticulum metallopeptidase 1
ENSBTAG00000015690	50574.71056	24792.09741	1.88E-05	PLIN4	perilipin 4
ENSBTAG00000015829	128.6740147	48.45479203	7.06E-05	ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)
ENSBTAG00000015844	1091.165284	2286.016647	0.00012817	TFPI2	tissue factor pathway inhibitor 2
ENSBTAG00000016071	17.35247576	52.08435641	0.00019537	HHIP	hedgehog interacting protein
ENSBTAG00000016185	635.226806	249.9922756	9.26E-05	ENAH	ENAH, actin regulator
ENSBTAG00000016612	1668.534121	757.5781036	0.00014287	NEK9	serine/threonine-protein kinase Nek9
ENSBTAG00000017069	449.7335938	150.098185	3.06E-06	FAM198B	family with sequence similarity 198 member B
ENSBTAG00000017183	481.9889959	1076.093889	0.0002451	PDLIM3	PDZ and LIM domain 3
ENSBTAG00000017567	41420.41051	11910.59833	1.48E-06	-//-	
ENSBTAG00000017811	973.620562	458.9769891	0.00026215	SLC39A9	solute carrier family 39 member 9
ENSBTAG00000017814	375.4529733	861.642656	3.18E-05	LGI4	leucine rich repeat LGI family member 4

ENSBTAG00000017860	1204.821143	349.3857997	8.23E-05	HIPK2	homeodomain interacting protein kinase 2
ENSBTAG00000018473	19.58660002	1.502643079	0.00021725	MARCO	macrophage receptor with collagenous structure
ENSBTAG00000018596	4114.720514	1809.355907	0.00029697	PTPN21	protein tyrosine phosphatase, non-receptor type 21
ENSBTAG00000018936	4874.427411	2245.589364	0.00025442	LSS	lanosterol synthase
ENSBTAG00000019146	49.78958759	189.1543759	0.00021501	OSBP2	oxysterol binding protein 2
ENSBTAG00000019181	292.8281455	78.18017565	0.00026904	BAIAP2L1	BAI1 associated protein 2 like 1
ENSBTAG00000019423	0.81944714	10.40662587	1.74E-05	-//-	
ENSBTAG00000019792	40.13390616	10.54248153	1.79E-06	SLC16A9	solute carrier family 16 member 9
ENSBTAG00000020127	19.73092644	2.106505282	0.0001708	TKTL1	transketolase like 1
ENSBTAG00000020510	34.60591613	5.150384738	2.46E-08	EPHA10	EPH receptor A10
ENSBTAG00000021077	0.146795505	480.3474362	1.40E-08	-//-	
ENSBTAG00000021201	118.5020032	39.05161595	7.59E-05	FAM126B	family with sequence similarity 126 member B
ENSBTAG00000021272	191.7908079	59.80583548	1.64E-05	ABCG1	ATP-binding cassette sub-family G member 1
ENSBTAG00000021846	14.74420116	2.844730792	0.00014296	CELSR3	cadherin EGF LAG seven-pass G-type receptor 3 precursor
ENSBTAG00000021879	6675.435059	3242.223013	9.04E-05	VCL	vinculin
ENSBTAG00000024272	57.34609064	202.6382997	3.87E-05	-//-	
ENSBTAG00000026156	73.40221686	23.67674309	0.00012993	VGLL3	transcription cofactor vestigial-like protein 3
ENSBTAG00000026586	19.93731473	59.43686	0.00012022	PPP1R14C	Bos taurus protein phosphatase 1, regulatory (inhibitor) subunit 14C (PPP1R14C), mRNA
ENSBTAG00000027477	114.7434722	238.1988715	0.00028404	B3GAT1	galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1

ENSBTAG00000027654	2192.044386	5928.113361	7.97E-05	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1
ENSBTAG00000027727	0.307444632	10.96070206	1.03E-05	-//-	
ENSBTAG00000031014	157.5461527	68.81413347	0.00011314	LDAH	lipid droplet associated hydrolase
ENSBTAG00000033222	36.35171756	11.39967699	7.91E-05	CALR3	calreticulin 3
ENSBTAG00000034185	5757.145843	11502.16639	1.37E-05	-//-	
ENSBTAG00000034222	190.0880086	83.56420309	4.76E-05	CAB39L	calcium binding protein 39 like
ENSBTAG00000035959	894.8049265	2485.362411	8.74E-05	-//-	
ENSBTAG00000038116	2088.388599	978.8673035	4.61E-05	TGOLN2	trans-golgi network protein 2
ENSBTAG00000038128	101.0001041	519.8998552	1.36E-06	-//-	
ENSBTAG00000038865	87.09306416	406.6140231	7.39E-06	TCEA3	transcription elongation factor A protein 3
ENSBTAG00000039231	11122.08267	4669.785231	0.00028924	MTURN	maturin, neural progenitor differentiation regulator homolog
ENSBTAG00000039520	96.46826651	473.6272009	0.00025263	-//-	
ENSBTAG00000039817	1069.271446	75.9976309	0.00014606	-//-	
ENSBTAG00000039935	111.0402338	39.44186874	1.66E-05	KCNC3	potassium voltage-gated channel subfamily C member 3
ENSBTAG00000040128	3974.305284	1644.647695	5.79E-06	FZD4	frizzled class receptor 4
ENSBTAG00000043969	23.88666748	2.293306998	7.85E-05	CALN1	calcium-binding protein 8
ENSBTAG00000045728	113910.7939	31860.2529	0.00011489	-	acyl-CoA desaturase
ENSBTAG00000046026	16.86312941	3.244113821	2.98E-05	SLC39A4	solute carrier family 39 member 4
ENSBTAG00000046671	5150.242679	2248.832526	2.30E-06	WFS1	wolframin ER transmembrane glycoprotein
ENSBTAG00000046753	1682.520522	673.1060365	0.00018151	-//-	
ENSBTAG00000047129	374.6071213	172.3349006	0.00020174	B3GNT6	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6
ENSBTAG00000047202	126.5940365	49.01673285	8.74E-06	GRIN1	glutamate ionotropic receptor NMDA type subunit 1

ENSBTAG00000047547	63.7939157	499.0909109	3.26E-10	-//-	
ENSBTAG00000047664	198.6901685	77.48955824	3.29E-05	GNAZ	G protein subunit alpha z
ENSBTAG00000047957	39888.46882	10364.76124	4.15E-05	-//-	
Novel00106	125.50364	53.69306147	5.84E-05	--	hypothetical protein,,conserved [Babesia bigemina]
Novel00310	515.3340076	40.37445809	4.92E-06	--	PREDICTED: mitogen-activated protein kinase 7-like isoform X2 [Ovis aries musimon]
Novel00311	64.06872708	7.426433227	5.04E-07	--	PREDICTED: DNA repair protein complementing XP-C cells isoform X1 [Ovis aries]
Novel00315	258.4548246	101.1330095	0.00013823	--	PREDICTED: uncharacterized protein LOC101123602 isoform X1 [Ovis aries]
Novel00370	50.92050676	9.59530524	0.00027891	--	PREDICTED: protein FAM47E isoform X9 [Ovis aries musimon]
Novel00465	218.1937502	16.58386705	0.00023285	--	PREDICTED: protoheme IX farnesyltransferase, mitochondrial-like [Bos mutus]
Novel00589	67.85807695	18.5634269	1.28E-05	--	syncytin-Rum1 precursor [Bos taurus]
Novel00611	66.40055527	26.59275255	9.39E-05	--	PREDICTED: putative uncharacterized protein FLJ22184 [Bos taurus]
Novel00662	46.30600986	103.7545322	0.0003035	--	PREDICTED: fibrocystin-L isoform X3 [Bos taurus] TPA: novel gene vertebrate polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1 (PKHD1L1)-like protein [Bos taurus]
Novel00803	897.3970069	1850.714789	9.41E-05	--	PREDICTED: uncharacterized protein LOC102395992 [Bubalus bubalis]

Novel01179	233.5112005	77.89174265	2.16E-06	--	hypothetical protein M91_10594, partial [Bos mutus]
Novel01199	68.25399124	15.2735876	5.96E-09	--	TPA: transmembrane 9 superfamily member 2 [Bos taurus]
Novel01269	55.30672969	127.8568747	0.00013063	--	TPA: ring finger protein 213-like [Bos taurus]
Novel01461	88.02558727	632.2555556	4.30E-06	--	PREDICTED: putative uncharacterized protein FLJ22184 [Bos taurus]
Novel01525	18.60714702	4.441029387	0.00015045	--	PREDICTED: EF-hand calcium-binding domain-containing protein 1 isoform X5 [Ovis aries musimon]
Novel01923	32.22964516	105.4103234	3.63E-07	--	hypothetical protein M91_02473, partial [Bos mutus]
Novel01999	19.55536118	4.641204659	0.00017314	-//-	
Novel02234	140.4474547	66.11668422	0.00014913	--	TPA: cyclin-dependent kinase 14 [Bos taurus]
Novel02316	72.66487907	20.54470245	4.64E-05	--	hypothetical protein, conserved [Babesia bigemina]
Novel02346	168.3267559	74.99438722	4.69E-05	--	endonuclease/reverse transcriptase [Sus scrofa]
Novel02474	37.75122363	12.80167563	5.61E-05	--	bitter taste receptor Bota-T2R65A [Bos taurus]
Novel02718	173.4143411	64.12296984	1.13E-05	--	hypothetical protein, conserved [Babesia bigemina]
Novel02809	107.9620241	45.63412071	5.48E-05	--	hypothetical protein, conserved [Babesia bigemina]

Table 4. All Standard to Choice muscle tissue differentially expressed genes

Gene code	Standard reads	Choice reads	P-value	Gene abbreviation	Gene name
ENSBTAG00000000670	30.88305	246.4221	1.80E-07	RRP9	ribosomal RNA processing 9, U3 small nucleolar RNA binding protein
ENSBTAG00000000706	677.3053	290.5012	3.38E-05	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif 1
ENSBTAG00000002191	117.7992	1902.022	2.91E-10	RRP12	ribosomal RNA processing 12 homolog
ENSBTAG00000002362	503.0079	156.3534	2.52E-07	APOLD1	apolipoprotein L domain containing 1
ENSBTAG00000003981	55.35508	344.0517	8.49E-07	RRP1	ribosomal RNA processing 1
ENSBTAG00000005146	417.5045	120.3775	1.79E-05	-//-	
ENSBTAG00000008993	105.2256	32.21649	4.91E-05	INTS6L	integrator complex subunit 6 like
ENSBTAG00000009656	25.03987	3.93732	7.16E-07	-//-	
ENSBTAG00000009838	13.50673	205.7908	1.28E-07	-	RRP15-like protein
ENSBTAG00000012451	186.654	72.58464	5.64E-05	-//-	
ENSBTAG00000015735	1.99076	138.0085	1.02E-09	-//-	
ENSBTAG00000015844	625.1162	252.1213	7.12E-05	TFPI2	tissue factor pathway inhibitor 2
ENSBTAG00000016656	147.7579	32.84557	3.46E-05	PARP14	poly(ADP-ribose) polymerase family member 14
ENSBTAG00000017412	91.89222	215.9969	1.50E-05	SOCS6	suppressor of cytokine signaling 6
ENSBTAG00000017418	57.82568	213.2538	5.76E-05	RRP1B	ribosomal RNA processing 1B
ENSBTAG00000017527	91.55428	31.19422	6.73E-06	CRYBG1	crystallin beta-gamma domain containing 1
ENSBTAG00000018638	35.41229	11.35007	5.69E-05	CC2D2A	coiled-coil and C2 domain containing 2A

ENSBTAG00000018773	117.0008	40.52153	1.55E-05	RND1	Rho family GTPase 1
ENSBTAG00000019054	84.35166	27.60798	2.48E-06	EPSTI1	epithelial stromal interaction 1
ENSBTAG00000019822	1413.131	3930.522	1.45E-05	TPPP3	tubulin polymerization promoting protein family member 3
ENSBTAG00000021308	600.7675	1307.075	3.38E-05	IRS1	insulin receptor substrate 1
ENSBTAG00000025274	2842.782	6437.747	4.23E-05	TUBB4B	tubulin beta 4B class Ivb
ENSBTAG00000031829	104.1807	304.4241	2.02E-06	-//-	
ENSBTAG00000032369	106.2415	38.89256	2.79E-05	NMI	N-myc and STAT interactor
ENSBTAG00000032588	48.75623	15.35005	3.82E-05	TMEM150C	transmembrane protein 150C
ENSBTAG00000038652	1553.892	634.9172	2.69E-06	-//-	
ENSBTAG00000043582	254.2079	2460.898	4.44E-06	-//-	
ENSBTAG00000047501	0	99.71181	7.82E-05	bta-mir-2887-2	bta-mir-2887-1
Novel00172	2705.266	1094.076	7.27E-05	-//-	
Novel01032	4133.773	1110.92	6.64E-05	--	PREDICTED: UPF0545 protein C22orf39 homolog isoform X1 [Bos taurus]
Novel02175	24.28261	6.572796	5.17E-05	--	PREDICTED: SLAM family member 5-like isoform X4 [Bos taurus]
Novel02197	102.023	8.821389	7.62E-07	--	PREDICTED: guanylate-binding protein 4 isoform X1 [Bos taurus]
Novel02218	2303.582	5734.034	3.21E-06	--	PREDICTED: kelch-like protein 30 isoform X2 [Bos taurus]
Novel02423	13.05624	1.721383	2.31E-05	--	hypothetical protein, conserved [Babesia bigemina]
Novel02900	64.25278	2181.705	1.31E-07	-//-	

Table 5. All Select to Choice muscle tissue differentially expressed genes

Gene code	Select reads	Choice reads	P-value	Gene abbreviation	Gene name
ENSBTAG00000006519	111.6178	39.07056	4.01E-06	TNFRSF25	TNF receptor superfamily member 25
ENSBTAG00000014217	84.99102	20.37391	7.17E-06	HHEX	hematopoietically expressed homeobox
ENSBTAG00000015727	205.4899	41.14806	2.31E-06	-//-	
ENSBTAG00000046264	257.6161	70.05945	3.94E-06	-//-	
Novel01279	15.14206	55.62733	7.24E-07	--	hypothetical protein, conserved [Babesia bigemina]