Factors affecting intramuscular adipose tissue development in beef cattle.

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Introduction

The U.S. beef industry has been utilizing growth enhancing technologies in daily management practices due to market incentives to increase rate of weight gain and efficiency of growth in cattle. Two of the most widely used growth enhancing technologies are steroidal implants and beta-adrenergic agonists. Growth-promoting implants have been approved for use in cattle during the suckling, growing, and finishing stages of production, for over 50 years. Implants have been utilized to improve growth rates of implanted cattle by 30%, feed efficiency by 15% (Preston, 1999), and carcass leanness by 8% (Johnson et al., 1996; Bruns et al., 2005) as compared to nonimplanted controls. The use of a single implant that contains both estrogen (estradiol) and androgen (trenbolone acetate, TBA), increased feed efficiency and leanness over either substance alone (Preston, 1999). In 2007, Reinhardt conducted a review on the economic return of different implant programs. The researcher found that an aggressive implant program can have a net value of $85.68 per head over nonimplanted cattle (Reinhardt, 2007). In order to help maximize quality grade, Reinhardt (2007) suggested using a stair-step implant program to help maximize quality which can have a net value of $103.35 per head over nonimplanted cattle. Beta-adrenergic agonists cause increased lipolysis, increased protein accretion, or decreased lipogenesis (Mersmann, 1998). Supplementation of beta-adrenergic agonists in diets can lead to increased rate of gain, feed efficiency, leanness, and dressing percentage (Moody et al., 2000). Along with implants, the increase in weight gains and feed efficiency following the use of beta-adrenergic agonists can help increase profitability in beef cattle operations.

While the benefits of implants and beta-adrenergic agonists have been well documented, growth promotants generally shift nutrient utilization toward carcass lean tissue deposition at the expense of adipose tissue. Due to this shift, concerns about negative impacts of growth promotants on quality grade and tenderness have developed in the industry. Numerous researchers have concluded that the use of growth promotants can compromise beef carcass quality grades due to reduced marbling scores (Belk and Cross, 1988; Morgan, 1991; Belk, 1992 as cited by Duckett et al., 1997; Morgan, 1997; Roeber et al., 2000). Duckett et al. (1996) reviewed 37 implant trails and detected mean reductions of 24% in marbling scores and a 14.5% reduction in the number of carcasses grading choice with implants. Researchers have also determined that more aggressive implant strategies or the repetitive use of implants may be detrimental to beef carcass quality (Morgan, 1997; Roeber et al., 2000). The 1991 and 2000 National Beef Quality Audits identified “reduced quality of beef due to implants” as a main concern for the packing industry (Smith et al., 1992; Roeber et al., 2000). The 2005 National Beef Quality Audit reported that 23.6% of carcasses had marbling scores greater than or equal to

Small[50] (Garcia et al., 2008) which is 36.6% less than what was reported in a 2000 audit by McKenna et al. (2002).

While the impact of growth promotants on economic returns is generally positive, a reduction in quality grade can lead to loss of potential dollars within the industry. Roeber et al. (2000) surveyed purveyors, retailers, and restauranteers and discovered that 64%, 55%, and 97%, respectively use at least some beef product that is low Choice or higher. In addition, it is well known that as consumers' disposable income increases, people tend to buy higher quality protein products as a part of their daily diets. As the world's population increases and as countries become more economically developed, it is important that U.S. beef producers are capable of providing more total pounds of lean beef, as well as, high quality beef products. This paper will highlight the process of intramuscular adipose tissue deposition in beef cattle and how different growth promoting compounds can affect the development of intramuscular adipose tissue in finishing beef cattle.

Intramuscular adipose tissue development in beef cattle

The chemical composition of a carcass is affected by several factors, including, sex, genetic background, body weight (BW), plane of nutrition and age. Fattening occurs as a normal part of growth and as an animal begins to approach market weight. It is believed that selecting for lean meat yield is antagonistic to marbling. Hammond (1932) and McMeekan (1940) first characterized that the sequence of tissue depot development from earliest to latest is skeleton, muscle, and fat. Berg and Butterfield (1968) confirmed this same order of development in cattle. The order of deposition of adipose tissue is intermuscular, subcutaneous, and intramuscular (Andrews, 1958). Intramuscular adipose tissue, also known as interfascicular or marbling adipose tissue contributes the least to total carcass fat and can be distinguished from other adipose fat depots by its location within the perimysium which surrounds the bundles of muscle fibers (Moody and Cassens, 1968). Numerous researchers have reported that intramuscular fat content increases with increasing days on feed (Dolezal et al., 1982; Miller et al., 1987; Schroeder, 1990; Duckett et al., 1993; Alderson, 1994; Bruns et al., 2005), but plateaus as time is increased (Moody et al., 1970; Butts et al., 1980; Greene et al., 1989; May et al., 1992; Van Koevering et al., 1995). Research by Bruns et al. (2005) reported that fractional growth rate for intramuscular adipose tissue decreased more than fractional growth rate for whole carcass fat or protein, which may indicate that intramuscular adipose tissue, is not strictly a late developing depot, but has the potential to develop early in the feeding periods.

Adipose tissue is a connective tissue that is derived from the mesoderm of the developing embryo. These mesenchymal cells give rise to an early form of fat cells known as adipoblasts. Since adipose tissue is responsible for storing energy for the body, during early adipose tissue development there is an increased vascularization of connective tissue which may be associated with the development of an extensive capillary network. After early vascularization, a collection of adipoblasts form lobules that will eventually form a larger lobe encased in a sheath of

collagenous fibers. Adipoblasts will continue to proliferate until a variety of cells send signals to stop replicating. At this time, adipoblasts may differentiate into preadipocytes. Otto and Lane (2005) have described how mesodermal cells form committed preadipocytes/adipofibrobasts in a cell culture model. As lipid droplets form in the preadipocyte, they merge to become one large globule which is characteristic of a mature adipocyte. Mature adipocytes are the predominant cell type in adipose tissue.

The enlargement of adipose tissue within an animal can occur by hyperplasia (increase in cell number) or hypertrophy (increase in cell size through lipid accumulation). As an animal grows and fat is deposited, a population of cells accumulate lipid and increase in volume and diameter. Additional cells may be recruited to adipose tissue depots to aid in growth of the tissue (Singh et al., 2007). Hood (1982) proposed that the increase in adipocyte number may be due to preadipocytes filling with lipid and becoming large enough to count or through actual differentiation or proliferation of newly stimulated adipocytes. Fernyhough et al. (2005) demonstrated in cell culture models that mature adipocytes containing considerable lipid may still proliferate.

Smith and Crouse (1984) have demonstrated that for intramuscular adipose tissue development glucose contributes a greater proportion of acetyl units to fatty acid biosynthesis than in subcutaneous adipose tissue. In intramuscular adipose tissue, glucose contributed approximately 70% of the acetyl units to fatty acid biosynthesis, whereas acetate and lactate contributed less than 20% of the acetyl units. However, in subcutaneous adipose tissue glucose contributed less than 5% to total acetyl units. More recent investigations have shown that in long-fed steers, lipid synthesis from acetate was depressed even though marbling scores increased over time (Chung et al., 2007). These data indicated that glucose likely replaced acetate as the primary substrate for fatty acid biosynthesis in intramuscular adipose tissue.

Transdifferentiation

Several in vitro studies have demonstrated a role for peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT-enhancer binding protein (C/EBPβ), and stearoyl-Co A desaturase (SCD) in regulating adipogenic gene expression. Proliferator-activated receptor gamma and C/EBPβ are involved in both the differentiation of adipocytes (Umek and McKnight, 1991) and the transdifferentiation of myoblasts to adipoblasts (Hu, 1995). SCD helps regulate fatty acid composition of lipids in adipose tissue. Poulos and Hausman (2006) and Torii et al. (1998) reported that PPARγ and C/EBPβ increased adipocyte number in the skeletal muscle of cattle and swine. When myoblasts are exposed to thiazolidinedione (TZD), both PPARγ and C/EBPβ are expressed in the transdifferentiation process (Figure 1). Transdifferentiation from myoblasts to adipocytes can be induced by TZD (Hu et al., 1995; Teboul et al., 1995; Grimaldi et al., 1997; DeCoppi et al., 2006). Mukherjee et al. (2000) reported that TZD activates GLUT4 which works to improve insulin sensitivity and glucose uptake. As mentioned in the previous section, glucose is the primary substrate for intramuscular adipose tissue development. Thus,

TZD is important for enhancing marbling and the quality of beef cattle. TZD can also transdifferentiate bone marrow cells (Gimble et al., 1996) and fibroblast-like cells (Torii et al., 1998) to adipocytes. Singh et al. (2007) investigated the effects of ciglitizone, a potent TZD, on transdifferentiation of porcine muscle satellite cells to adipoblasts. Exposure of the porcine muscle satellite cells to ciglitizone weakened the formation of fused myotubes and increased the formation of cells containing lipid droplets. The cells that were treated with ciglitizone, showed an increase in expression of PPARγ and C/EBPβ.

Chung and Johnson (2009) reported that treatment of bovine satellite cells (BSC) with insulin, oleic acid, ciglitizone, estradiol-17β, and melengestrol acetate (MGA) lead to an increase in lipid droplets in single cells and multi-nucleated myotubes as compared to the control treatment. In addition, PPARγ mRNA expression tended to increase and C/EBPβ significantly increased in cells that had estradiol-17β and MG treatment. Chung and Johnson (2009) also reported that the treatment of C_2C_{12} myoblast and 3T3-L1 preadipocyte cultures with MGA lead to increases in C/EBPβ mRNA expression. These studies indicate that under the appropriate stimulation, populations of various cells in the cell culture model can express adipogenic genes and accumulate lipid.

Impact of steroidal implant hormones on adipogenesis in beef cattle

Studies have shown that increases in lean tissue accretion have resulted in decreased intramuscular adipose tissue deposition (Van Barneveld, 2003; Schwab et al., 2006; 2007). Both indirect and direct mechanisms have been proposed as potential reasons marbling decreases while lean tissue increases. An indirect mechanism was proposed by Duckett et al. (1999) and is the dilution effect. The dilution effect is the belief that intramuscular marbling content stays the same across implanted and nonimplanted animals, however, due to an increase in ribeye area size the amount of marbling seems smaller (Duckett et al., 1999). In terms of energy, implantation leads to increased energy needs in an animal. Since adipose tissue is a lower priority than muscle tissue, additional nutrients or energy will be directed towards lean tissue accumulation and away from adipose tissue. The combination of energy partitioning and the dilution effect may be why implanted animals have lower marbling scores.

More recent research has shown the direct effect of implants on adipogenic gene expression. Genes such as C/EBPβ, PPARγ, and SCD play essential roles in adipose tissue development throughout an animal's life. Research by Chung et al. (unpublished) and Singh et al. (2003) have shown a decrease in mRNA levels of these adipogenic genes when cattle are implanted. The reduction in gene expression could be another reason why implanted cattle have decreased intramuscular adipose tissue.

Research in a cell culture model has helped gain a better understanding of the mode of action for implants. Postnatal muscle growth in beef cattle occurs through hypertrophy since muscle fiber number is fixed at birth. In order for hypertrophy to occur, satellite cells are needed to fuse with existing fibers and contribute their nuclei to provide the fibers with the DNA necessary to support the increased size of the muscle fiber (Moss and Leblond, 1971; Swatland, 1977; Campion, 1984). Allen et al. (1979) reported that 60 to 90 percent of the DNA in mature muscle fibers come from satellite cells (Figure 2). Since satellite cells are essential for muscle growth, their proliferation and fusion can be a critical rate limiting step in muscle growth. In adult animals, the number of satellite cells decrease and the remaining satellite cells are in a non-proliferative state or quiescence. Johnson et al. (1998) reported that bovine satellite cells (BSC) cultured from steers implanted with a combination implant proliferated more rapidly than satellite cells from their nonimplanted counterparts. Thus, implants work by activating quiescent cells which enhances muscle growth. Additionally, studies by Kamanga-Sollo et al. (2004) have shown that insulin-like growth factor I (IGF-I) mRNA levels increased in proliferating BSC treated with estrogen or trenbolone for 48 hours. IGF-I is also known to increase protein synthesis and decrease protein degradation. These two studies support the mechanism that implants induce muscle growth through increased production of IGF-I which then activates quiescent satellite cells leading to increased muscle growth. The effects of MGA or progesterone (P4) in cell culture is opposite of the results found for implants. Sissom et al. (2006) reported that the addition of MGA or P4 resulted in a decrease in DNA synthesis as measured by [^{3}H]-thymidine incorporation in BSC and C_2C_{12} myoblasts. MGA causes a reduction in ribeye area and these studies suggest how carcass composition may be affected through a decrease in satellite cell proliferation. Interestingly, at the same time as MGA decreased muscle cell proliferation, it upregulated adipogenic gene expression.

As discussed earlier, steroidal implants improve daily gain and feed efficiency on average in implanted cattle as compared to nonimplanted controls. However, there have been numerous research studies showing a decrease in marbling score and quality grade as a result of implants. Duckett and Andrae (2001) showed that a single estrogen implant or an estrogen/androgen combination implant reduced marbling score by 4% and increased ribeye area by 3 to 4%. Other researchers have also found that marbling score was decreased in cattle that were implanted (Belk, 1992; Herschler, 1995; Milton et al., 1996; Morgan, 1997; Duckett, 1997, 1999; Mader, 2000; Roeber et al., 2000). This reduction in marbling was further exacerbated in cattle that received more than one implant. Cattle that were reimplanted had decreased marbling scores by 6 to 11% and increased ribeye area by 4 to 8 % (Duckett and Andrae, 2001). Bartle, Herschler, Cooper et al., Mader et al., Roeber et al., and Platter et al., have found similar results when reimplanting with a combination implant halfway through the finishing phase (1992; 1995; 1999; 1999; 2000; 2003). In contrast, Johnson et al. (1996), Gerken et al. (1995), Pampusch et al. (2003), and Smith et al. (2007) have shown that implants have no effect on quality grade.

In a review by Duckett et al. (1997) of 77 steer and 30 heifer research trials, there was a significant decrease in marbling score and % Choice (except in androgen-only implants) in once implanted steers compared to controls regardless of implant type. Marbling scores and quality grade in heifers were not significantly impacted. In reimplanted heifers, marbling scores were reduced by estrogen and androgen combination implants. Implanting with combination implants resulted in the largest improvements in DMI, gain, feed efficiency, and ribeye area as compared to estrogen or androgen implants, but also had the greatest decrease in percent choice (17%). These results indicated that there is a relationship between reduced marbling score and increased ribeye area. Some researchers have found that implanting cattle in the suckling or growing phase did not have a negative impact on carcass quality (Mader, 1994; Kuhl, 1997; Schaneman and Pritchard, 1998; George et al., 2000). In contrast Hardt et al. (1995) showed that administrating four implants over different time periods of an animal's life reduce quality grade by 9%.

A summary of implant usage by Duckett and Andrae (2001) showed an inverse relationship between marbling score and longissimus area. Implanted cattle grow faster, but they do not accumulate adipose tissue at a rate proportional to muscle tissue. Cattle that are harvested at a lower fat-content would also have a lower marbling score. Preston et al. (1990) proposed that steers and heifers that receive a combination implant need an additional 12 and 15 days, respectively, on feed to have marbling scores equal to their nonimplanted counterparts. Gerken et al. (1995) and Smith et al. (2007) showed no impact on longissimus crude fat percentages in cattle that were implanted versus nonimplanted controls. In contrast, Foutz et al. (1997) and Duckett et al. (1999) found a decrease in total fatty acid percentage was decreased. However when Duckett et al. (1999) accounted for changes in the ribeye size, implanting did not alter the total fatty acid percentage. These studies show that by feeding implanted cattle longer, any decrease in marbling score could be overcome.

In addition to the performance and carcass data that has been collected on cattle, researchers have studied the impact of implants on gene expression in cattle. Genes such as PPARγ and C/EBPβ are expressed during the early and middle stages of feeding and SCD is a later marker for adipocyte differentiation. Chung et al. (unpublished, Figure 3) showed that relative mRNA levels of PPARγ, C/EBPβ, and SCD increased during days on feed. However, implanting cattle causes a decrease in adipogenic gene expression. There was no difference in C/EBPβ mRNA levels with different implants, but PPARγ and SCD mRNA levels were lower with combination implants. Smith et al. (2007) also revealed combination implants inhibited markers of adipose development. Singh et al. (2003) used the pluripotent, immortalized cell like C3H, 10T1/2 to show testosterone and dihydrotestosterone increased myogenic cells and decreased adipocytes, while down regulating PPARγ and C/EBPβ expression. A decrease in adipogenic gene expression when implants are utilized may help explain why marbling is reduced. The adipogenic gene expression data, combined with performance and carcass data, provides the cattle industry with a better understanding of how steroidal implants impact

intramuscular adipose development in beef cattle and what management practices may be used to overcome these hurdles.

Impact of β-adrenergic agonists on adipogenesis in beef cattle

While β-adrenergic agonists only have minimal direct effects on intramuscular adipose tissue development in beef cattle, there is the potential for indirect effects to occur due to enhanced muscle hypertrophy and fiber type changes. Figure 4 illustrates the proposed mechanism of β-adrenergic agonists on adipogenesis and myogenesis. Skeletal muscle is composed of a heterogeneous population of muscle fibers that have the ability to contract at different speeds and metabolize energy in different ways. There are three muscle fiber types in the bovine skeletal muscle, myosin heavy chain (MHC) type I, IIA, and IIX, which are determined by their contractile speed (Chikuni et al., 2004). Muscle fibers are classified as either oxidative, glycolytic, or both oxidative and glycolytic due to energy metabolism (Pette and Staron,1990). Myosin heavy chain type I isoforms are slow twitch fibers due to their use during aerobic work and reliance on oxidative metabolism to supply energy. Myosin heavy chain type IIA and IIX are known as fast twitch fibers due to their use during anaerobic work and reliance on both oxidative and glycolytic metabolism to supply energy. The supplementation of beta-adrenergic agonists can lead to the shift of the metabolic characteristics or phenotypes of muscle fibers (Johnson, 2004). Numerous studies have reported that beta-adrenergic agonists can causes a shift in the amount of isoforms expressed, shifting from slower, oxidative types to faster, glycolytic fiber types, with an increase in the size of type IIB fibers (Beermann et al., 1987; Zeman et al., 1988; Polla, 2001). The shift in fiber types can also affect the animal's ability to accumulate marbling. Research has shown that muscles that contain increased glycolytic fiber types will have less intramuscular fat than muscles with more oxidative fiber types (Melton et al., 1974, 1976; Calkins et al., 1981).

In swine, ractopamine has been shown to cause a decrease in slow fiber types and an increase in MHC type IIB fiber types (Depreux et al., 2002). Zeman et al. (1988) and Polla et al. (2001) have reported that rats fed clenbuterol exhibited a transition from slower, aerobic fiber types to faster, anaerobic fiber types. Kim et al. (1987) reported that lambs fed cimaterol did not affect the proportion of type I to type II fibers in the longissimus or semitendinosus, but type II had 50% greater cross-sectional area in both the longissimus and semitendinosus muscles. There was no effect on the cross-sectional area of type I fibers (Kim et al., 1987). In cattle, Baxa et al. (2010) reported a tendency for zilpaterol hydrochloride (ZH) to decrease MHC type IIA fiber types and increase MHC type IIX. Further investigation by Kellermeier et al. (2009) showed that ZH supplemented cattle had greater fiber diameters. This is similar to the report by Miller et al. (1988) that showed an increase in diameter of type II fibers in steers administered clenbuterol. Vestergaard et al. (1994) reported that in cimaterol-fed bulls there was a conversion in proportion of MHC type IIA to IIB isoforms with hypertrophy of MHC type IIB and not MHC type IIA isoforms.

These results show that beta-adrenergic agonists induce a shift from slow twitch fibers to fast twitch fibers following administration. It has been shown that MHC isoforms composition contributes to the variations observed in postmortem energy metabolism, susceptibility to adverse pH, temperature effects, and meat quality in porcine muscle (Bowker et al., 2004). This shift of MHC isoform expression can result in an impact on bovine skeletal muscle meat quality as well.

Conclusions

Growth promoting agents such as implants and beta-adrenergic agonists will continue to be used in the beef feedlot industry to increase feed efficiency and growth rates, which in turn improves profit margins. Numerous in vitro and in vivo studies have been conducted, that have increased our understanding of the mechanism of action of these agents at increasing protein accretion and/or decreasing protein degradation in beef cattle. Additionally, studies have shown that the use of implants and beta-adrenergic agonists can have negative impacts on intramuscular adipose tissue development both in a direct and indirect manner. Mitigation of the reduced marbling caused by growth promotants would allow greater net value of these products in the beef industry.

Researchers have found that cells within muscle can be stimulated to transdifferentiate towards an adipocyte lineage with proper treatment. These findings indicate that with the proper agents and management practices, producers can work to eliminate the negative quality effects of implants or beta-adrenergic agonists. Reinhardt (2007) reported that with the proper implant program, implanted cattle can have a net profit of $103.35 per head over nonimplanted cattle. As the demand for high quality beef continues to increase it is vital that producers find a management strategy that will allow them to be efficient in producing more pounds of high quality beef.

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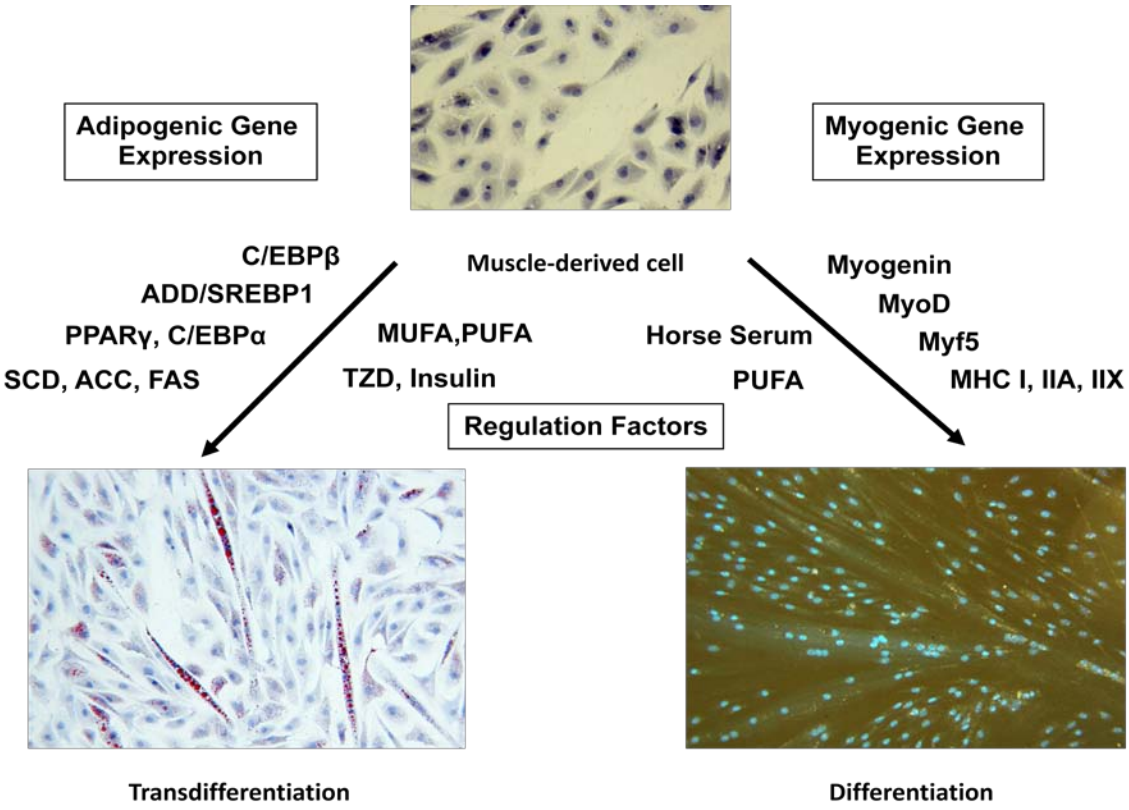

Figure 1. The role of transcription factors and adipogenic genes related to bovine satellite cell transdifferentiation. Transcription factors regulated by specific ligands are involved in triggering adipogenic differentiation and expressing functional adipogenic genes. Transdifferentiation could convert muscle satellite cells to preadipocytes.

Figure 2. Bovine satellite cells have been shown to be critical in supporting postnatal muscle growth. Under steroidal implant stimuli, these cells can undergo cell division and will fuse into the existing fiber, thus donating their DNA to support skeletal muscle hypertrophy. However, the existing adipocytes have lower levels of stored triglycerides.

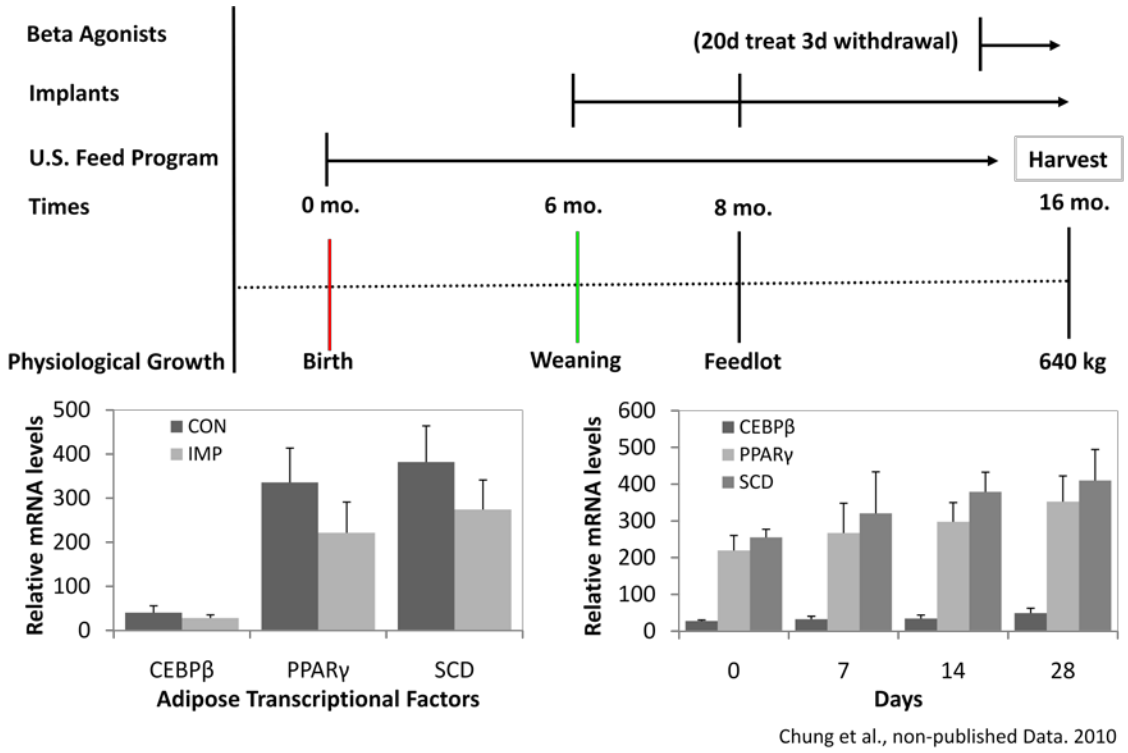

Figure 3. Timeline of beef cattle feeding program in U.S. CCAAT/enhancer binding protein β, peroxisome proliferator activated receptor γ, and stearoyl CoA desaturase mRNA abundance in bovine LM tissue in yearling steers on feed. Biopsy samples were collected on d 0, 7, 14, and 28 on feed from 20 individual steers. Total RNA was isolated from skeletal muscle tissue and relative C/EBPβ, PPARγ, and SCD gene expression was determined using real-time quantitative-PCR. Relative C/EBPβ, PPARγ, and SCD gene expressions were increased throughout the 28 day trial. However, muscle biopsies from implanted cattle had lower adipogenic gene expression than those from nonimplanted cattle.

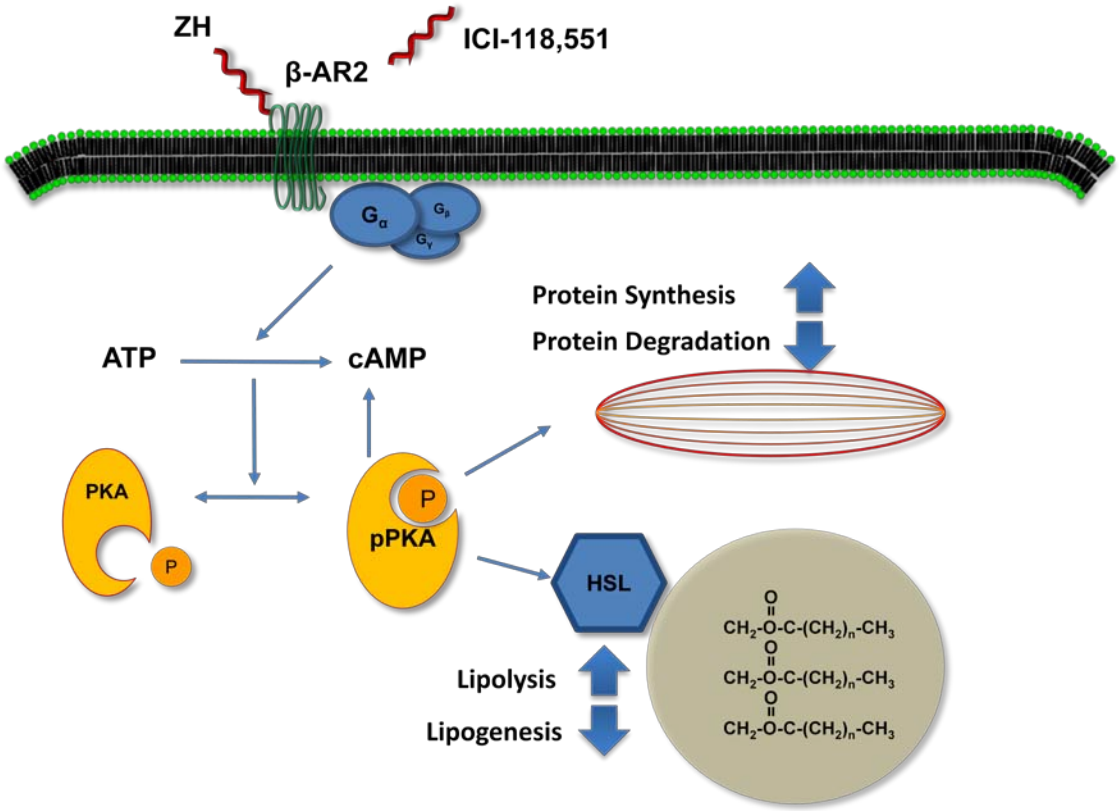

Figure 4. The proposed mechanism of β-adrenergic agonists on adipogenesis and myogenesis in cultured cells.