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Review

## Intercellular signaling between adipose tissue and muscle tissue

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### Abstract

Adipose and muscle tissues undergo regulated growth and differentiation processes that are modulated by a wide range of factors. The interactions between myogenic cells and adipocytes play a significant role in growth and development, including the rate and extent of myogenesis, muscle growth, adipogenesis, lipogenesis/lipolysis, and in the utilization of energy substrates. Important hormones and growth factors involved in the regulation of these processes include glucocorticoids, insulin-like growth factors, various cytokines, insulin, and leptin. Interactions among these axes have important implications in their influence on relative fat and lean deposition and the efficiency of energy utilization in growth and development. As research progresses to better clarify the interactions among adipose tissue depots and muscle of different fiber types, pathways will become better understood, ultimately leading to the optimized management of fat and lean growth in domestic livestock species. This review will focus on elements of intercellular signaling, using data from cell culture studies to illustrate specific examples of signaling pathways between cells.

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### 1. Introduction

Growth and development of fat and muscle are regulated by means of complex and incompletely understood mechanisms of communication among cells [1,2]. Adipocytes undergo a development and differentiation process in which preadipocytes, which are fibroblast-like

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cells, transform into lipid-filled fat cells [1]. During differentiation, different markers are expressed as the cell reaches its terminal morphological stage [2,3]. Important regulators of the differentiation process include insulin, glucocorticoids, and many other hormone axes [2,3].

Muscle tissue and cells also undergo a regulated growth and differentiation processes, and these, as well as substrate utilization and energy partitioning, are also affected by a range of factors [4,5]. Recently, the (signaling) interaction between myogenic cells and adipocytes has been implicated as playing a significant role in the rate and extent of adipogenesis, myogenesis, and lipogenesis/lipolysis [3,6,7]. Key factors in these processes include leptin, insulin-like growth factors, and adiponectin [7]. Leptin and leptin binding proteins, by direct actions and interactions with other hormones, are thought to play an important role in the communication between adipocytes and myogenic cells [7,8].

This review will focus on elements of intercellular signaling, particularly those involving leptin and leptin associated proteins, which mediate pathways directed toward domestic animal myogenesis, adipogenesis and lipogenesis/lipolysis. Where possible, data from cell culture studies have been used to illustrate specific examples of signaling pathways between cells. Several recent reviews provide coverage of elements of this discussion [1–5,7–12]. These provide a useful background, some of which has been framed in the present discussion to support data from recent experimental reports.

## 2. Adipose tissue

### 2.1. Adipose growth and differentiation

#### 2.1.1. Steps of differentiation

The first step in adipogenesis is the commitment of progenitor cells to the adipocyte lineage, after which they cannot revert back to a less differentiated “stem-like” cell [3,13]. Once committed, adipoblasts undergo an exponential replication phase that terminates and the cell cycle arrests at G1. Early markers of differentiation, such as lipoprotein lipase (LPL), are then expressed, and these cells, known as preadipocytes, may then undergo further proliferation [3]. After preadipocytes stop proliferating, late markers of differentiation, such as glycerol-3-phosphate dehydrogenase (GPDH) and fatty acid synthetase (FAS) are detected. Cells then begin lipid accumulation in the cytosol at which time cells are called adipocytes [3]. Terminal adipocyte differentiation is seen as the accumulation of lipid so that the nucleus is displaced from the center to the periphery of the cell [3]. Adipocytes have also been reported to dedifferentiate, a process where mature adipocytes lose their lipid filled morphology and characteristics and assume a fibroblast-like appearance, regaining preadipocyte characteristics [14–17]. Recently we have found that bovine adipofibroblasts undergo spontaneous dedifferentiation in culture (Figs. 1 and 2, unpublished data). This characteristic suggests that specific signals that have yet to be identified may prove useful in manipulating carcass composition. The ability to induce lipid loss and dedifferentiation in specific adipose depots would provide a powerful tool to regulate composition and meat quality.

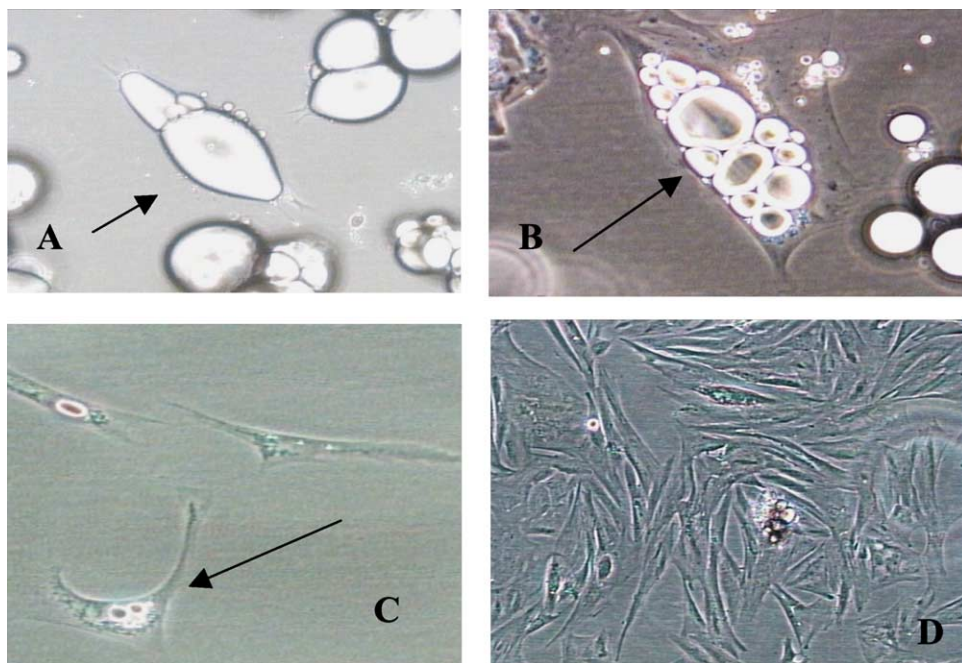


Fig. 1. Phase contrast photomicrographs of isolated fat cells in a variety of stages of development in vitro. (A) Mature fat cells in ceiling culture (arrow; 20 $\times$ ). (B) Multilocular fat cell reverting to an adipofibroblast (arrow; 40 $\times$ ). (C) Adipofibroblasts that are beginning to proliferate (arrow; 20 $\times$ ). (D) Proliferating adipofibroblasts (10 $\times$ ).

### 2.1.2. Gene expression during adipocyte differentiation

During differentiation of preadipocytes to adipocytes [3], preadipocytes undergo changes in morphology as well as gene expression. Peroxisome proliferator activated receptor  $\gamma$  (PPAR- $\gamma$ ), PPAR- $\alpha$ , and CCAAT/enhancer binding protein factor (C/EBP)  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\zeta$  are important transcription factors involved in the regulation of adipocyte differentiation [18–20]. Lee et al. [20] reported that in mice with induced hyperleptinemia, expression of PPAR- $\alpha$  is increased in white adipose tissue, and may therefore be a modulator of leptin action, causing a reduction in fat by the up regulation of carnitine palmitoyl trans-



Fig. 2. Fat cells stained with oil-red-o to demonstrate lipid. (A) Mature fat cell in ceiling culture (arrow; 40 $\times$ ). (B) Cells losing lipid at six days in culture (arrow; 40 $\times$ ). (C) Cells reverting to adipofibroblasts (20 $\times$ ).

ferase (CPT-1) and acyl CoA oxidase (ACO). PPAR- $\gamma$  is activated prior to the activation of C/EBP- $\alpha$  and is associated with preadipocyte differentiation [18]. The inhibitory effects of retinoids on differentiation of adipocytes is believed to be mediated through PPAR- $\gamma$  and C/EBP- $\beta$  [3]. C/EBP- $\beta$  transactivates C/EBP- $\alpha$  expression, promoting adipocyte differentiation. C/EBP- $\alpha$  binds the promoter region of the adipose-specific genes such as leptin and adipocyte lipid binding protein (aP2) [3,18,19]. Also expressed is GLUT4, an important insulin-mediated glucose transporter in adipocytes, as is the case in muscle. aP2 (also known as A-FABP) is an intracellular fatty acid binding protein that is expressed during differentiation along with fatty acid binding protein (FABP) and fatty acid transferase (FAT), and these molecules are responsible for the transport of fatty acids into the adipocyte and subsequent lipid accumulation [3,21]. C/EBP- $\alpha$  is necessary for triglyceride accumulation and adipocyte differentiation. C/EBP- $\alpha$  knockout mice die shortly after birth because they are unable to accumulate fat [19]. C/EBP- $\alpha$  also inhibits proliferation of adipocytes. C/EBP- $\zeta$  plays a role in the negative regulation of C/EBP- $\alpha$ , as does the growth factor *c-myc*, which blocks the induction of C/EBP- $\alpha$ , thereby inhibiting adipose conversion. Adipogenesis can also be inhibited by long chain polyunsaturated fatty acids, which, when taken up by the cell, act as transcription repressors, resulting in a reduction in C/EBP- $\alpha$  and PPAR- $\gamma$  [3]. Table 1 summarizes the range of markers expressed during adipocyte differentiation.

### 2.1.3. Regulation of adipose differentiation by glucocorticoids, insulin, and insulin-like growth factors

Glucocorticoids, insulin, and insulin-like growth factors (IGFs) are all involved in the regulation of adipocyte proliferation and differentiation [3,22–24]. Glucocorticoids activate C/EBPs, showing another possible mechanism by which differentiation is regulated [19]. Glucocorticoids act through the glucocorticoid receptor (GR), resulting in an allosteric change which enables the hormone receptor complex to bind the glucocorticoid response element (GRE), the classical glucocorticoid promoter, and modulate transcription [25]. Additionally, Floyd and Stephens [25] reported that signal transducer and activator of transcription 5A (STAT 5A) interacts with the GR during adipogenesis, resulting in inhibition of adipocyte differentiation, indicating a potential regulatory role in adipocyte gene expression. In an in-vitro study in human stromal vascular cells, Hauner et al. [23] reported that cortisol or insulin alone promoted little differentiation, but the addition of cortisol with insulin resulted in 47% increase in differentiation. The naturally occurring steroid prednisolone and the artificial steroid dexamethasone increased differentiation by up to 67% over the control, indicating the importance of glucocorticoids and insulin in the conversion of preadipocytes into mature fat cells. However, other studies have reported cell proliferation and differentiation without the use of dexamethasone, so it may not be a required component of adipocyte media [16,26,27]. Furthermore, in times of stress, other hormones, such as epinephrine and ACTH are released, and these stimulate lipolysis, supplying an available energy source [28].

Insulin-like growth factors (IGFs) also regulate adipogenesis, providing an example of a paracrine interaction between skeletal muscle and adipose tissue. Smith et al. [29] reported that IGF-I is essential for preadipocyte differentiation into adipocytes, although it is not clear if the mechanism of action is mainly through the Type 1 receptor or the insulin receptor pathway. It was demonstrated by Jia and Heersche [22] that dexamethasone stimulated the

Table 1  
Markers expressed during preadipocyte/adipofibroblast differentiation into adipocytes

Factor	Time expressed	Effect	Reference
AD3	Early	Preadipocyte recruitment	Yu and Hausman [176] Hausman and Richardson [177]
ADD1/SREBP1	Early	Stimulates PPAR- $\gamma$ , transactivates leptin and FAS	Kim et al. [178]
CEBP- $\beta$	Early	Transactivates CEBP- $\alpha$ , promotes differentiation, activates PPAR- $\gamma$	Lee et al. [19]  Boone et al. [3] Tang et al. [179] Sorisky [2]
CEBP- $\delta$	Early	Activates PPAR- $\gamma$ and CEBP- $\alpha$	Boone et al. [3]  Sorisky [2]
CEBP- $\zeta$	Early	Negative regulation of CEBP- $\alpha$	Lee et al. [19]
FAT	Early	Fatty acid transport, lipid accumulation	Boone et al. [3]
LPL	Early	Fatty acid metabolism	Boone et al. [3] Sorisky [2]
Pref-1	Early	Inhibits differentiation	Lee et al. [180] Mei et al. [181]
PPAR- $\gamma$	Mid	Preadipocyte differentiation, activates GLUT4	Kersten et al. [18]  Yamamoto et al. [182] Sorisky [2]
CEBP- $\alpha$	Mid	Binds promoter region of leptin and AP2, inhibits proliferation	Lee et al. [19]  Sorisky [2]
Adipsin	Late	Terminal differentiation	Diamond [6]
AP2	Late	Intracellular fatty acid binding protein, lipid shuttle	Hansen et al. [46]  Han et al. [183]
GLUT4	Late	Glucose transport	Sorisky [2]
Leptin	Late	Terminal differentiation	Diamond [6]
GPDH	Late	Triacylglycerol accumulation	Ailhaud [184] Sorisky [2]
$\alpha_2$ -Adrenoceptor	Late	Anti-lipolytic	Saulnier-Blache et al. [33]
HSL	Late	Triacylglycerol release	Sorisky [2]

proliferation of preadipocytes in the presence of IGF, but dexamethasone or IGFs alone were unable to stimulate differentiation, suggesting that both glucocorticoids and IGFs may be required for differentiation *in vitro*, and the IGF responsiveness of adipocyte progenitors is a result of dexamethasone stimulation. The addition of an anti-insulin-like growth factor-1 receptor (IGF-1R) antibody had no effect on differentiation, thus it was proposed that the IGF-mediated stimulation of adipocytes acts through a different signal transduction pathway than the IGF-1R pathway. Thus, this effect is probably mediated via the insulin receptor. Consistent with the data reported in muscle, Boney et al. [30] reported that IGF-1 stimulates both the proliferation and differentiation of preadipocytes via the IGF-1 receptor which ultimately leads to activation of mitogen activated protein kinase (MAP-K). MAP-K inhibition stimulates preadipocyte differentiation, and there is a decrease in MAP-K in cells in the latter stages of differentiation, similar to what is observed in muscle. The loss of MAP-K activity in differentiating cells was a result of the loss of Shc and not IRS-1. Consequently, it was concluded by Boney et al. [30] that the IGF-1 signaling switch from proliferation to differentiation is a result of the switch from Shc to IRS-1 mediated signaling. It is noted, however, that the aforementioned studies were performed in 3T3-L1 cell lines which may behave differently from primary cell lines [31].

#### 2.1.4. Regulation through adrenoceptor signaling

Lipolysis is typically controlled by intracellular cyclic AMP (cAMP), a key signal cascade intermediate for which intracellular levels are at least partly regulated by a range of guanosine 5'-triphosphate (GTP)-linked receptors [32]. Activated adrenoceptors coupled to GTP-binding proteins regulate adenylyl cyclase levels, thereby controlling cAMP [32]. Adipocytes express both  $\alpha_2$  and  $\beta$  adrenoceptors (AR) [33]. Stimulation of  $\beta$ -AR induces the activation of adenylyl cyclase by the stimulatory GTP binding protein,  $G_s$  [33]. This results in an increase in cAMP levels and the phosphorylation of hormone sensitive lipase (HSL) by cAMP dependent protein kinase A (PKA), inducing lipolysis [33]. In contrast, activation of the  $\alpha_2$ -AR inhibits adenylyl cyclase via the inhibitory GTP binding protein,  $G_i$ , resulting in the counteraction of the lipolytic effect of the  $\beta$ -AR [33]. Saulnier-Blache et al [33] reported that expression of the  $\alpha_2$ -AR emerged late during preadipocyte differentiation in hamster cells, and expression is dependent on the age and size of the fat cell, thus the  $\alpha_2$ -AR may also be a useful marker of differentiation.

#### 2.1.5. Cytokine superfamily members

Adipocyte differentiation is also influenced by members of the cytokine family, including TNF- $\alpha$ , TGF- $\beta$ , IL-11, GH, and leptin [3]. TNF- $\alpha$ , TGF- $\beta$ , and IL-11 are all inhibitors of adipocyte differentiation, and can cause the reversal of the adipocyte phenotype [30,35]. TGF- $\beta$  decreases differentiation by decreasing C/EBP- $\alpha$  and PPAR- $\gamma$ , but the effect may be reversed by dexamethasone [34]. IL-11 was reported to inhibit adipogenesis and lipoprotein lipase activity in 3T3-L1 adipocytes, and this was controlled at a posttranscriptional level, possibly by the means of a tyrosine phosphorylation pathway [35].

**2.1.5.1. TNF- $\alpha$ .** Gasic et al. [36] demonstrated that TNF- $\alpha$  stimulates lipolysis by blocking endogenous adenosine that is spontaneously released from cells. Adenosine typically binds to receptors and activates  $G_i$ , inhibiting adenylyl cyclase, thereby decreasing intracellu-

lar cAMP and decreasing the rate of lipolysis [36]. TNF- $\alpha$  signaling blocks this pathway by down regulating  $G_i$ , consequently resulting in an increased rate of lipolysis in adipocytes [36]. TNF- $\alpha$  was additionally reported to down regulate many adipocyte genes, such as adiponectin, CCAAT/EBP- $\alpha$ , and PPAR- $\gamma$ , thereby having a negative effect on adipocyte differentiation [37]. Also, TNF- $\alpha$  has been implicated in the induction of serine phosphorylation of IRS-1, causing the inhibition of the insulin receptor tyrosine kinase, attenuating the actions of insulin and implicating it in a potential role of insulin resistance [38–40]. Consistent with these data, TNF- $\alpha$  may also down regulate GLUT4 in adipocytes and muscle, resulting in reduced intracellular glucose uptake [6]. Furthermore, Lofgren et al. [41] reported TNF- $\alpha$  had no effect on basal glucose uptake, but inhibited insulin-stimulated glucose uptake, also supporting this interaction between the two hormones. Similar to cell culture studies, it was reported that injection of heifers with recombinant bovine TNF- $\alpha$  resulted in the inhibition of glucose stimulated insulin secretion and insulin-stimulated glucose utilization, suggesting metabolic effects of this cytokine in vivo resemble in vitro effects [42]. Leptin appears to have many similar actions to TNF- $\alpha$ , favoring lipolysis over lipogenesis, and has also been implicated in interactions with insulin, which will be discussed later in this review.

**2.1.5.2. Growth hormone.** Glucose uptake by fat cells is also regulated by growth hormone (GH). GH is strongly lipolytic, inhibiting glucose uptake, resulting in a net decrease in stored lipids [7]. GH promotes the differentiation of 3T3-L1 preadipocyte cell lines [27,43] while inhibiting the differentiation of primary rat and human preadipocytes [44]. Tominaga et al. [45] demonstrated that the effect of GH was determined by the stage of differentiation of the preadipocytes. GH administered early after induction of differentiation promoted differentiation, but when added later when the cell was beginning to accumulate lipid, differentiation was inhibited. Hansen et al. [46] reported that in primary rat preadipocytes, GH reduced PPAR- $\gamma$  expression by 47%, but had no effect on early adipocyte markers such as adipocyte determination and differentiation factor (ADD1), a factor that activates LPL and FAS. However, GH inhibited late markers of differentiation, aP2, FAS, and hormone-sensitive lipase (HSL), by 67–77%. Hansen et al. [46] reported the mechanism to be independent of MAP-K pathways, and hypothesized the mechanism of inhibition to be via the preadipocyte factor 1 (Pref-1)/fetal antigen 1 (FA1) mechanism, as GH prevents a typical decrease in these factors that occurs during differentiation. Paradoxically, GH may also enhance adipose differentiation by acting through an IGF-1 pathway, stimulating gene expression of the IGF-1 receptor, thereby enhancing the mitotic effects of IGF-1, increasing the number of recruited preadipocytes available for differentiation [3]. However, the IGF-corticosteroid interaction cited above may be a more likely activator of IGF action in this case than is GH. Additionally, Lissett et al. [47] reported that treatment with GH caused an initial increase, then decrease, in circulating leptin levels, possibly indicating the presence of a negative feedback loop.

## 2.2. Factors produced by fat

### 2.2.1. Leptin

Leptin is a 16-kDa protein hormone produced primarily by white adipose tissue as the expression product of the Ob gene [8,47–55]. The primary role of leptin is in the regulation of energy flow [47,48,50,52,55,56]. Providing biological linkages to this important

function, leptin is also produced by numerous tissues and cells, including brown adipose tissue [57], fetus [58], placenta [52,59,60], skeletal muscle [61], pituitary gland [62,63], bone marrow [64], liver [65], stomach [66], mammary gland [67] and fibroblasts [51]. The central and peripheral actions of leptin ultimately result in a decrease in energy conservation, and increasing body energy utilization, partitioning substrates toward oxidation rather than storage [8,55,68–72]. Leptin gene expression increases in response to feeding, elevated blood glucose and glucocorticoid levels, and is reduced in response to fasting, insulin deficiency, and high cAMP levels [70]. A deficiency in either leptin production or receptor availability results in animals exhibiting obesity, hyperphagia, hyperinsulinemia and other metabolic disorders [72].

### 2.2.2. Central actions of leptin

Leptin is found in the central nervous system (CNS), and apparently serves to influence energy expenditure and possibly food intake [47,55]. Leptin levels in the CNS are influenced by the interaction between leptin and CNS-specific hormones such as neuropeptide Y and melanin-concentrating hormone and its receptor [73,74]. In addition, leptin has been shown to alter circulating glucocorticoid levels via effects on the hypothalamic-pituitary-adrenal axis [75]. Leptin regulation in the CNS is similar to that exerted by interleukin-1 (IL-1), which has led Luheshi et al [55] to suggest that IL-1 may mediate some of the actions of leptin.

### 2.2.3. Leptin receptors

Both long and short isoforms of the leptin receptor exist [59,76]. Isoforms differ at the C-terminus (intracellular domain), but all share the same 783 amino acid extracellular ligand-binding domain [8]. The long form of the receptor, termed ObR<sub>b</sub>, primarily in rodents [54] or ObR<sub>L</sub> in other animals [71], is located in the hypothalamus and is thought to be responsible for mediating the central effects of leptin on hormone release. Short receptor isoforms, termed Ob-R<sub>a</sub>, c, d, and e in rodents [59,71] are widely distributed throughout peripheral tissues. Ob-R<sub>a</sub> in rodents, also termed OB-R<sub>S</sub> in other species, is the predominant short receptor isoform and its specific function has yet to be clearly elucidated [8,71,76].

Leptin interaction with the long receptor isoform is propagated as follows. Leptin binds to the receptor causing receptor dimerization, which then induces the activation of JAK. The JAKs phosphorylate tyrosine sites on the receptor, which act as docking sites for STATs, that then dimerize and are translocated to the nucleus where they interact with DNA and activate transcription [8,71]. Additionally, JAK activation by leptin may lead to the activation of IRS-1 and MAP-K [8].

The leptin receptor (Ob-R), is a member of the class I cytokine receptors. The OB-R is structurally similar to gp121, the common signal-transducing component of the IL-6 cytokine family [77]. Nakashima et al. [77] determined that OB-R and gp121 mediate overlapping but distinct signaling pathways. Because of similar receptors and signaling pathways, leptin shares functional similarities with the cytokines TNF- $\alpha$  and IL-6, which act to decrease LPL activity and inhibit GLUT4 [6,7]. All three hormones are regulated similarly [55], their interactions having a further layer of complexity as TNF- $\alpha$  also stimulates leptin production [7].

#### 2.2.4. Leptin/adipocyte interactions

Leptin may possess autocrine capabilities with mature adipocytes. In this scenario in fat cells, leptin might be synthesized by well-nourished adipocytes to prevent adipocytes from internalizing more lipid [32]. Fruhbeck et al. [32] reported that the ability of leptin to stimulate lipolysis was a result of its interference with the adenosine A<sub>1</sub> receptor. Leptin signaling apparently reduces the A<sub>1</sub> receptor interaction with the adenylyl cyclase G<sub>i</sub> protein, thereby removing the inhibition and increasing lipolysis in adipocytes [32]. These same authors reported no effect on isoproterenol-induced lipolysis, acting through the  $\beta$ -AR signal via G<sub>s</sub>, suggesting no interaction via catecholamine induced lipolysis [32]. Additional autocrine effects may be a result of the interactions between leptin and adenosine. Adenosine is a nucleoside released by cells that acts as an extracellular signal via specific receptors that couple to guanine nucleotide binding proteins. Activation of the G<sub>i</sub> coupled A<sub>1</sub>- (the most prevalent form in adipose tissue) or A<sub>3</sub>-receptor decreases cAMP levels, while activation of the G<sub>s</sub>-coupled A<sub>2</sub> receptor increases cAMP [78]. Rice et al. [78] reported that adenosine activation of the receptor stimulated leptin secretion, and suggested this to be a possible mechanism whereby an increase in circulating leptin levels may counteract the anti-lipolytic effects of adenosine. Similarly, Cheng et al. [79] reported an increase in leptin secretion with the stimulation of adenosine, and adenosine release by adipocytes was stimulated by insulin, providing a mechanism for insulin-stimulated leptin release in adipocytes.

#### 2.2.5. Adiponectin

Another hormone secreted by adipocytes that has a role in energy homeostasis regulation and influence on insulin signaling pathways is adiponectin, also known as adipocyte complement related protein (Acrp28) [80–82]. Adiponectin is an abundant adipose-specific protein, and its levels are associated with obesity and insulin sensitivity [81]. The primary functions of adiponectin are to stimulate fatty acid oxidation, decrease plasma triglycerides, and improve glucose metabolism [82]. Wu et al. [80] reported that in rat adipocytes, adiponectin treatment increased AMP activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation (thus its inactivation), consequently increasing fatty acid oxidation. It was further determined that adiponectin enhanced glucose uptake in adipocytes without stimulating tyrosine phosphorylation of IR or IRS-1. AMPK inhibitors blocked adiponectin enhanced glucose uptake, indicating the mechanism of glucose uptake in adipocytes by adiponectin is different from insulin stimulated glucose uptake [80]. However, Stefan et al. [81] reported that adiponectin is associated with insulin sensitivity, and adiponectin increased insulin-induced tyrosine phosphorylation of IR in skeletal muscle, resulting in improved glucose tolerance. Although these reports are not consistent, this may be a result of different actions in different tissues.

### 2.3. Role of fat depots in fat metabolism

The depot from which the adipose tissue arises has major effects on its metabolism. Intramuscular fat deposition is apparently regulated by different factors than those regulating fat deposition in other tissue depots, and there are metabolic differences between intramuscular and subcutaneous fat depots [83]. Miller et al. [83] and May et al. [84]

reported that in cattle, intramuscular adipocytes are smaller than subcutaneous fat cells. Additionally, activities of the glycolytic enzymes hexokinase and phosphofructokinase were higher in intramuscular depots. Subcutaneous fat depots had higher levels of the lipogenic enzymes NADP-malate dehydrogenase, 6-phosphogluconate dehydrogenase, and glucose 6-phosphate dehydrogenase, indicating tissue depots had unique aspects of lipid metabolism. Furthermore, breed also appeared to be a factor in the lipogenic activity of the tissue depots [83,84].

Intermuscular fat depots had similar characteristics of intramuscular fat depots. Eguinoa et al. [85] reported that in cattle, the intermuscular fat depot had the smallest adipocyte size when compared to omental, peri-renal, and subcutaneous depots. The intermuscular depot additionally had a lower level of lipogenic enzyme activity than other depots, as was the case in intramuscular fat depots. However, when size was adjusted for, subcutaneous and intermuscular fat depots had higher enzyme activity than the other depots, indicating a potential role of other factors such as blood flow and lipolytic activity as determinants of depot differences observed [85].

There appears to be higher leptin mRNA levels in subcutaneous than omental fat depots, but this may be due to adipocyte volume. It has been reported that leptin is correlated to adipocyte volume, and subcutaneous adipocytes are larger than omental adipocytes [86].

#### 2.4. Energy partitioning in fat cells

LPL is involved in the movement of fatty acids from triacylglycerol in blood chylomicrons and very low density lipoproteins (VLDLs) to the adipocytes [28,87,88]. This transport is augmented by insulin and glucose [28]. Hormone Sensitive Lipase (HSL), which is regulated by catecholamines and ACTH, has the opposite effect, mobilizing fatty acids from adipocytes into the bloodstream in the process of lipolysis. Leptin also has a lipolytic effect, mobilizing adipocyte fatty acids into the bloodstream [8]. Acylation stimulating protein (ASP) is a protein synthesized in the cytosol of adipocytes that has an important role in adipogenesis [89]. ASP stimulates fatty acid uptake and synthesis, promoting movement of glucose transporters to the cell membranes by activation of a diacylglycerol–protein kinase C pathway [90]. If glucose is present in high enough concentrations, it may provide an increased amount of substrate available for the synthesis of fatty acids and triglycerides [7,91]. ASP also has an influence on leptin. In two separate studies, Murray et al. [92,93] reported decreased leptin levels in male [92] and female [93] ASP knockout mice. There were also decreased levels of adipose tissue mass, and basal glucose and insulin levels, leading the authors to suggest an indirect effect on leptin via changes in plasma insulin and glucose [93]. Clearly, disruption of lipid accumulation would have a negative influence on leptin expression and its concentration in the plasma. Jankowski et al. [102] reported that when diabetic patients were treated with sulfonylurea, a compound used to improve glycemic control, there was an increase in ASP of an equal magnitude to the decrease in plasma leptin level and suggested a potential cross talk between the two hormones. Because ASP is a product of the complement protein C3a, factor B, and adipsin [94], and adipsin expression has been found to be impaired in ob/ob and db/db mice [95], it was suggested that leptin signaling might have an influence on ASP production.

### 3. Muscle

#### 3.1. Muscle growth and differentiation

##### 3.1.1. Early development

Vertebrate skeletal muscle development begins during early embryogenesis and continues until muscle fiber formation is completed [96,97]. Distinct cell lineages, including skeletal muscle precursor cells arise from mesodermal progenitor cells in response to specific signals derived from surrounding tissues [96]. Myogenesis is controlled by both positive and negative extrinsic and intrinsic factors, allowing for the formation of a muscle progenitor cell (MPC) population [97], which is responsible for prolonged recruitment of muscle tissue during embryogenesis. Skeletal muscle cells are derived from the deromomyotome (DM), portion of the somite [97]. Cells of the DM lineage express Pax-3, Pax-7, Myf-5, myogenic basic helix–loop–helix (bHLH) factor, and MyoD [97]. These cells proliferate, and at the dorsolateral and ventrolateral edges of the DM, cells migrate to form the myotome, a sheet of proliferating skeletal muscle cells that express high levels of MyoD and Myf-5 [97]. Cells from the lateral DM migrate and eventually form the limb and express muscle-specific genes such as MyoD and Myf-5 [97,98]. The first muscle fibers that appear are multinucleated myofibers known as primary fibers [98,99]. About the time that innervation becomes established in the embryo, secondary myoblasts differentiate to form secondary fibers [98], which lie parallel to primary fibers, and are the predominant fiber type during the latter stages of embryogenesis. Secondary fibers tend to develop into fast muscle fibers, whereas primary muscle fibers tend to become slow fibers [98].

##### 3.1.2. Postnatal development

Satellite cells are another population of myogenic cells, and play a role in postnatal skeletal muscle growth, damage repair, and maintenance of adult skeletal muscle fibers [99]. The actual origin of satellite cells is not known, but it is thought that they originate from generic myogenic cells, specific myoblast lineages, or remnant embryonic or fetal myoblasts [100]. Satellite cells reside (trapped) between the sarcolemma and basal lamina of myofibers, and comprise only a small percentage of all muscle-related nuclei at birth [99]. Satellite cells are involved in the postnatal growth of skeletal muscle [101]. After puberty, satellite cells are mitotically quiescent until a stimulus, such as exercise or injury, induces the release of growth factors that stimulate the cells to re-enter the cell cycle [96,97,99,102]. Once into the cell cycle, satellite cells undergo multiple rounds of division forming a pool of myogenic precursor cells, which ultimately undergo terminal differentiation and fuse to growing myofibers to form multinucleated myotubes [96,97,99,102].

As is the case during early development myogenic regulatory factors, such as Myf-5, MRF-4, myogenin, and MyoD are involved in the activation, proliferation and differentiation of satellite cells [96]. An accelerated growth rate and delayed terminal differentiation was found in a culture of satellite cells isolated from mice lacking MyoD [96]. However, it was determined in-vivo that mice lacking MyoD or Myf-5 develop normal musculature, but mice lacking both MyoD and Myf-5 have no muscle development, indicating a redundancy of the genes for development of normal skeletal muscle. Poor postnatal development and death are also seen in mice lacking Pax-7, a factor expressed in both active and quiescent satellite

cells [96,97]. The processes of and factors required for skeletal muscle development have been extensively reviewed [97–99,101,103–108].

### 3.1.3. Factors produced by muscle tissue and cells

Muscle produced factors are under active investigation. Those factors that have been the most extensively studied are insulin-like growth factors (IGFs), insulin-like growth factor binding proteins (IGFBPs), and myostatin.

Myostatin is a factor secreted by muscle cells, which is involved in the regulation of muscle growth [42]. Myostatin acts as an inhibitor of muscle development via negative regulation of hyperplasia, resulting in limited number of muscle fibers and subsequent muscle size. Mutations of the myostatin gene results in increased muscle mass, and can result in double musculing in cattle [109]. Additionally, myostatin had been reported to negatively regulate satellite cell self-renewal, and signals satellite cell quiescence [110].

*3.1.3.1. IGF-1.* IGFs act as autocrine, paracrine, and endocrine factors produced by muscle and are crucial for muscle growth and development [111,112]. IGF-1 promotes both proliferation and differentiation of muscle cells [111,114–117]. Dodson et al. [118] reported that skeletal muscle satellite cells underwent proliferation upon exposure to physiological levels of IGFs, and the findings supported the hypothesis that interactions among growth factors play an important role in the control of postnatal muscle growth. Greene and Allen [119] reported that IGF-1 had no effect on proliferation but stimulated differentiation. However, when IGF-1 was combined with basic fibroblast growth factor, proliferation was increased and no differentiation was stimulated, indicating that proliferation and differentiation are regulated by interactions among growth factors [119]. Cheng et al. [120] demonstrated that inactivation of IGF-1 receptors decreased proliferation of muscle cells. Additionally, delayed expression of differentiation was associated with the prolonged phosphorylation of retinoblastoma, a protein that, when unphosphorylated, promotes the differentiation of skeletal myoblasts [120]. Therefore, IGF-1 signaling via IGF-1 receptors may be responsible for the timing and regulation of proliferation and differentiation. Tollefsen et al. [121] reported that both IGF-1 and IGF-1 receptors increase as the cell moves from proliferation to differentiation, suggesting a potential autocrine effect of IGFs.

Chakravarthy et al. [116] suggested that satellite cell proliferation was caused by IGF-I activating the PI3-K/Akt signaling pathway, initiating the progression of cells from the G<sub>1</sub> to S phase of the cell cycle by the down regulation of p27Kip1. This regulatory molecule maintains cells in the G<sub>1</sub> phase of the cell cycle, thereby inhibiting proliferation. However, other reports have indicated that the MAP-K pathway is important in cell proliferation while the PI3-K pathway is important during differentiation [113]. Two distinct signaling pathways of IGF-1 are reported to determine the proliferation and differentiation of muscle cells in one of these pathways. IGF-1 and IGF-2 bind to the IGF-1 receptor, causing a conformational change that induces receptor autophosphorylation and the phosphorylation of tyrosine on IRS-1. The phosphorylated tyrosines serve as docking sites for regulatory proteins, SHC or GRB2, which ultimately, through mammalian Son of Sevenless (mSos) and Ras, activate the Raf family of serine kinases, activating MEK which in turn activates the MAP-K (mitogen activated protein kinase) pathway. The subsequent activation of extracellular signal regulated kinase (ERK) stimulates proliferation and inhibits differentiation of skeletal

muscle cells [115,122–124]. In the other IGF mediated pathway, IRS-1 association with the PI3-kinase causes the phosphorylation and activation of the serine/threonine kinase P67 S6. Coolican et al. [115] suggested that these two pathways were distinct, as the inhibition of the MAP-K pathway repressed myoblast proliferation and induced differentiation while the inhibition of the P67 S6 kinase limited differentiation. However, Kaliman et al. [117] suggested that the p67 S6 kinase pathway is not involved in the actions of IGFs in rat, mouse, or human myogenic cells. Thus, despite great progress in investigation of IGF-1 mediated regulation, these processes still require further clarification.

**3.1.3.2. IGF-2.** Another important factor in the control of proliferation and differentiation of muscle cells is IGF-2. This peptide is produced by skeletal myoblasts in the early stages of terminal differentiation and is a critical survival factor in cells making the transition from proliferation to differentiation [125]. Kaliman et al. [126] reported that IGF-2 also induces terminal differentiation by activating a PI3-kinase pathway that initiates the degradation of I $\kappa$ B- $\alpha$ . This results in disassociation of I $\kappa$ B- $\alpha$  from nuclear factor- $\kappa$ B (NF- $\kappa$ B), which is then activated causing increased expression of inducible nitric oxide synthase (iNOS), factors. These factors are needed for this IGF-2-mediated mechanism resulting in terminal differentiation skeletal muscle cells [126]. Most of the actions of both IGF-1 and IGF-2 are mediated by the Type 1 IGF receptor, and at higher IGF concentrations, the insulin receptor can also be stimulated [127]. Thus, common signaling pathways are activated by all three peptides.

**3.1.3.3. Insulin-like growth factor binding proteins.** IGF induced differentiation and proliferation pathways are also regulated by insulin like growth factor binding proteins (IGFBPs) [111]. There are at least six different isoforms of the IGFBPs, which have about a 33% similarity with each other [112,128]. IGFBP 2, 4, 5, and 6 are expressed in skeletal muscle [12]. The major functions that have been proposed for IGFBPs are to act as transport proteins, to prolong IGF half-lives and regulate plasma clearance, to provide a means of tissue and cell localization, and to modulate interactions of IGFs with their receptors [127]. IGFBPs can either potentiate or inhibit the actions of IGFs, although IGFBP 4 has an exclusively inhibitory action [12,112]. IGFBPs have been reported to regulate IGF-mediated cell proliferation and myotube formation when expressed in large quantities, resulting in reduced availability of IGF-1 and IGF-2 [129]. Johnson et al. [130] reported that there were differing levels of IGFBPs in porcine myogenic cells between differentiated and non-differentiating cells, and between different phases of differentiation, indicating a role of these in the regulation of differentiation. The extensive literature describing IGFBP actions is not addressed further here. Comprehensive reviews are available [111,112,127,128].

## **4. The interaction between fat and muscle**

### *4.1. Fat as an energy storage and release organ*

Adipose tissue provides a large energy reserve that plays an important role in energy flux [7]. Adipocytes have a primary function to store energy during periods of excessive energy intake and to release it for metabolic processes during fasting periods or during high activity

[7,9]. This energy is primarily used by muscle. Moreover, adipose tissue acts as a secretory organ, secreting factors that are involved in maintaining energy balance and altering the dynamics of energy substrate utilization in response to varying physiological and metabolic demands [6,7,9].

#### 4.2. Depot effects on fat interaction with muscle

In addition to various regulatory factors, the adipose tissue depot also has an effect on adipocyte proliferation, differentiation, metabolism, and means of interaction with muscle. Some of these effects may be due to the fat depot's proximity to muscle, invoking different signaling pathways. For example, omental, mesenteric, and peri-renal fat depots are not in close proximity to muscle. Therefore, interactions between these depots and muscle can only be via endocrine mechanisms. In fat depots that are anatomically in close contact with muscle, such as subcutaneous, intermuscular, or intramuscular, signaling interactions may be endocrine or paracrine. Therefore, location may play a role in adipose tissue's physiological interactions, and the local interstitial milieu may manifest important determinants of the outcomes of these interactions.

#### 4.3. Muscle energy utilization

The major fuels of muscle are glucose, fatty acids, and ketone bodies. Rested muscle contains its own glycogen store, which can be converted into glucose 6 phosphate for entry into glycolysis. Glucose can also be obtained by mobilization of liver glycogen stores. Muscle also contains stores of TAGs that can be mobilized for energy use. Newly ingested lipids and carbohydrates are another energy source to muscle. Finally, muscle obtains energy from adipose release of fatty acids [131].

Maintenance of skeletal muscle metabolism requires considerable energy on the part of the animal, depending on fiber type and makeup of muscle mass [132]. Lipids, proteins and carbohydrates are utilized for energy, and substrate partitioning is dependent on complex interactions of a range of extrinsic and intrinsic factors. Temperature is one factor that plays a role in substrate utilization. Bell and Thompson [133] demonstrated that upon exposure to cold, there was an increase in plasma free fatty acids (FFAs) indicating an increase in lipolysis. This effect appeared to be regulated by the sympathetic nervous system, and was attenuated by feeding, indicating hormonal changes occurring after feeding can only partially counteract the sympathetic nervous system's fat mobilization effect [133]. Other extrinsic factors that affect substrate utilization include stress, nutritional status, age, sex, and exercise. Both endocrine and intracellular factors are important in controlling substrate balance throughout an animal's lifetime, and these will be discussed in the following sections [4,10,132].

There are three distinct and identifiable mechanisms that regulate energy substrate partitioning. First, substrate mobilization includes processes in adipose tissue, liver, and muscle, resulting in reversal of the energy storage processes gluconeogenesis, lipolysis, and glycogenolysis. These processes make the energy substrates glucose, FFAs and ketone bodies available. Second, substrate uptake includes groups of mechanisms by which substrates enter each cell type. Third, substrate oxidation is a highly regulated process that

determines which substrate will be utilized and their relative rates of utilization. It is important to note that in most physiological states, except heavy or prolonged exercise, the body's capacity for substrate uptake exceeds oxidation rate. Oxidation rate reflects demand of tissues. Therefore, regulation of oxidation rate is the critical factor of all three of the aforementioned mechanisms.

#### 4.4. Source of energy substrate

At rest and during low to moderate exercise (at  $VO_2$  max less than 47%) over a sustained period of time, lipids, in the form of blood borne free fatty acids of adipose origin, are the major substrate utilized by skeletal muscle [4,134]. During work, FFAs are mobilized from adipose tissue, resulting in an increase in the plasma concentration [5]. Consequently, muscle uptake and oxidation of FFAs increases, and plasma FFAs account for 77–90% of the muscle's lipid fuel requirements. The remainder comes from muscle FAs, evidenced by a decrease in the intramuscular store of triglycerides as exercise progresses [10].

Free fatty acids are derived from plasma nonesterified fatty acids (NEFA), circulating triacylglycerol fatty acids (TAGFA), and endogenously stored intramuscular TAGs [10]. The mobilization of FFAs is dependent on LPL and HSL, in mechanisms similar to those discussed previously in adipocytes [10,137]. HSL cleaves adipose TAGs to release NEFAs, and muscle localized HSL is responsible for the release of FFAs from intramuscular TAGs [10]. Muscle LPL cleaves the FFAs from TAGFAs and plays an important role in replenishing the muscle supply of TAGs depleted during exercise [10]. Both LPL and HSL have highest activity in oxidative muscle fibers [10].

#### 4.5. Fatty acid uptake

After fatty acids are mobilized by LPL and HSL, uptake across the plasma membrane occurs by diffusion or via transport proteins. At least three proteins are involved in the process, and their expression is greatest in oxidative fibers where fatty acid utilization is the highest [136]. The 40-kDa plasma membrane binding protein (FABP<sub>pm</sub>) mediates fatty acid transport across the plasma membrane [136]. Other FABPs are also thought to have a possible role in delivering fatty acids to the mitochondria and peroxisomes for oxidation [10]. There are multiple isoforms of FABPs present in different tissues, including FABP A which is found in adipose tissue, and FABP H which is found in multiple locations, including skeletal muscle [21]. An 84-kDa fatty acid translocase (FAT/CD36) mediates the short-term regulation of fatty acid uptake by muscle. During contraction, or during insulin stimulation via a phosphatidylinositol (PI3-K) signaling step, FAT/CD36 is translocated from an intracellular compartment to the plasma membrane, thereby increasing fatty acid uptake by muscle [134,135]. In addition, a 60-kDa fatty acid transport protein (FATP) has been identified, which also functions as a very long chain acyl-CoA synthetase that converts fatty acids to acyl-CoA, which may be esterified into TAG or undergo  $\beta$ -oxidation [10,136–139]. In both adipose tissue and skeletal muscle, it was reported that FATP expression was increased by PPAR- $\gamma$  and PPAR- $\alpha$  activators [140] and decreased by lipopolysaccharide, TNF, and IL-1 [141].

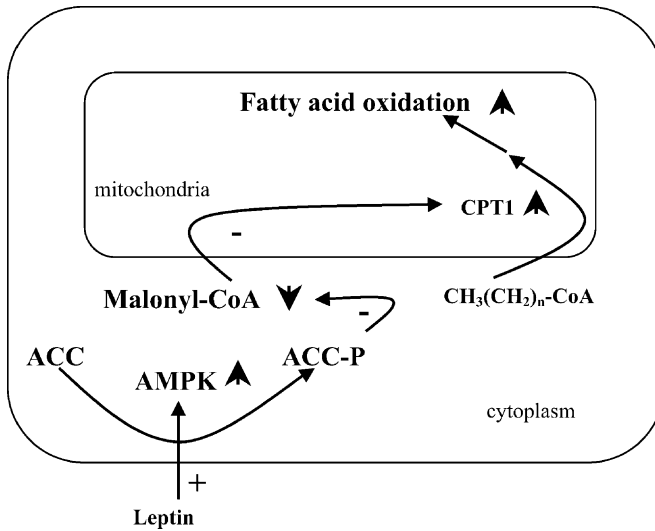


Fig. 3. In skeletal muscle, leptin stimulates AMPK, resulting in the phosphorylation of ACC, and the subsequent inhibition of malonyl-CoA. Decreased malonyl-CoA levels result in decreased inhibition of CPT-1, leading to increased fatty acid oxidation.

#### 4.6. Glucose transport in muscle

At high demand levels, glucose transport is the rate-limiting step of glucose utilization, and its primary transporter in muscle is the insulin-mediated glucose transporter 4 (GLUT4) [142,143]. Insulin mediates the translocation of the GLUT4 transporter to the cell surface. Insulin binds to the insulin receptor, a tyrosine kinase that invokes recruitment and phosphorylation of IRS-1. These proteins, when phosphorylated, act as docking proteins for PI3-K, which then activates Akt (protein kinase B), leading to the stimulation of GLUT4 translocation [144]. Tsao et al. [143] demonstrated that an increase in GLUT4 led to a decreased rate of FFA oxidation and increased glycogen synthesis and serum lactate levels, indicating the importance of glucose transport in the regulation of substrate utilization. For example, if the system is in a state of elevated glucose oxidation, it requires less FFA for the same level of demand.

#### 4.7. Energy partitioning in muscle

Partitioning between oxidative and biosynthetic pathways is at least partly regulated by AMPK, which is activated in response to low levels of ATP [145]. Winder and Holmes [145] reported that AMPK activation resulted in increased fatty acid oxidation and glucose uptake. Muoio et al. [138] determined that this enzyme inhibits *sn*-glycerol-3-phosphate acyltransferase, an enzyme involved in TAG synthesis. AMPK also phosphorylates and inactivates ACC (Fig. 3) [138,145].

Leptin also appears to be involved in this pathway, resulting in the repartitioning of fatty acids toward oxidation. Steinberg et al. [146] and Minokoshi et al. [147] reported that leptin

Table 2  
Similarities and differences between leptin and adiponectin action in muscle

	Adiponectin	Leptin
Fatty acid oxidation	Increase [149,150]	Increase [68,147]
AMPK activation	Increase [80,150]	Increase [146,147]
ACC phosphorylation	Increase [80,150]	Increase [146,147]
Glucose uptake	Increase [80,150]	Decrease [162]

treatment increased AMPK expression and phosphorylation in muscle, resulting in increased phosphorylation of acetyl-CoA carboxylase (ACC). ACC is an enzyme which catalyses the conversion of acetyl-CoA to malonyl-CoA [10,138,145]. Malonyl-CoA inhibits carnitine palmitoyl transferase-1 (CPT1), an enzyme involved in the regulation long-chain fatty acyl-CoA's transport into the mitochondria for oxidation. Upon inactivation of ACC by AMPK, malonyl-CoA levels decrease, resulting in decreased inhibition of CPT1, which causes an increase of fatty acid oxidation [10,138]. Bavenholm et al [148] reported that increases in glucose and insulin cause an increase in malonyl-CoA, resulting in a decrease of skeletal muscle fatty acid oxidation. The increase in malonyl-CoA is due to an increased concentration of citrate, an allosteric activator of ACC. Citrate also inhibits phosphofructokinase, an enzyme involved in glycolysis. Therefore, it was hypothesized that this pathway may prove to be a mechanism whereby glucose modulates its own use.

Another adipocyte-secreted hormone that interacts with muscle and may have a potential influence on insulin-signaling pathways and energy homeostasis regulation is adiponectin. It has been suggested that adiponectin accelerates fatty acid oxidation in muscle cells, reduces the plasma concentration of plasma fatty acids, and reduces hyperglycemia by improving insulin sensitivity [82]. Fruebis et al. [149] also reported that acute treatment of mice with adiponectin caused an increase in fatty acid oxidation in muscle, and a decreased level of plasma free fatty acids. In a study by Tomas et al. [150], it was reported that incubation of rat extensor digitorum longus muscle with adiponectin, or administration to mice *in vivo*, resulted in increased AMPK activation and ACC phosphorylation, indicating a potential pathway by which adiponectin increases skeletal muscle fatty acid oxidation in a manner similar to leptin. Table 2 summarizes similarities and differences between leptin and adiponectin-mediated energy substrate utilization.

PPAR- $\gamma$  is an important transcription factor with major roles in adipose tissue lipid metabolism. Lapsys et al. [151] determined that this factor was also important in skeletal muscle and plays a role in the expression of the genes LPL, mCPT1, and FABP, all involved in skeletal muscle lipid metabolism as outlined above, and is therefore an important transcription regulator present in skeletal muscle.

#### 4.8. Exercise effects on substrate utilization

During exercise above 47%  $\text{VO}_2$  max the major fuel source of skeletal muscle switches toward carbohydrates rather than fatty acids [134]. The length of time that an animal is able to maintain exercise above 47%  $\text{VO}_2$  max depends on the animal's condition and is species specific. Typically,  $\text{VO}_2$  max is ten times the resting metabolic rate, but in some species,

such as canines and equines, it is 20–28 times resting metabolic rate. Increased  $\text{VO}_2$  max allows for a greater aerobic rate, consequently extending the time prior to exhaustion [5]. As exercise intensity increases, substrate utilization moves from fat oxidation to oxidation of blood borne glucose to oxidation of glycogen. However, in production species, a negligible proportion of energy use occurs above 47%  $\text{VO}_2$  max. Hocquette et al. [5] reported that glucose turnover increases as the level of exercise rises, and the proportion of glucose which is directly oxidized in sheep can rise from 18% at rest up to 47% during exercise, with the remainder of the energy being supplied by NEFAs. Upon exercise at or above 47–57%  $\text{VO}_2$  max, there is an increase in the levels of blood glucose released from liver glycogen stores that are mobilized for energy. Pethick et al. [152] proposed that, during sustained exercise, blood glucose concentration increases, and lactate and pyruvate levels increase during the first fifteen minutes, but then fall below pre-exercise values, indicating low Cori-cycle activity.

In the Cori cycle, lactate produced by muscle glycolysis is transported via the blood to the liver and used for gluconeogenesis, providing glucose that can be utilized by the heart or returned to the muscle [131].

#### 4.9. *Insulin/leptin effects on substrate utilization*

Insulin is an important factor in the determination of substrate utilization. Jacob et al. [153] reported that insulin inhibited lipolysis in both skeletal muscle and adipose tissue. Dyck et al. [154] determined that insulin increased the uptake of fatty acids by inducing the translocation of the FAT/CD36 receptor to the plasma membrane. In the mouse, insulin was also reported to reduce FFA oxidation and increase FA incorporation into TAG in skeletal muscle, apparently via a PI3-K pathway [155]. Furthermore, Muoio et al. [155] reported that insulin decreased oleate oxidation by 29% and increased oleate incorporation into triglycerides by 43%, demonstrating the insulin effect of favoring muscle lipid storage over catabolism. However, it appears that insulin's effect on lipid partitioning is secondary to its effect on glucose uptake [155]. Furthermore, it appeared that increasing glucose uptake in muscle inhibited muscle fatty acid oxidation.

Insulin cross-talk with leptin and subsequent effects on energy partitioning may provide a means by which fat and muscle interact. The intersection of the leptin and insulin pathways is supported as an increase in fatty acid oxidation in muscle associated with insulin resistance appears to be linked to an increase in diacylglycerol synthesis and activation of protein kinase C [156–158]; a reduction in insulin-stimulated IRS-1-associated PI-3-activity, a blunting of insulin-stimulated IRS-1 tyrosine phosphorylation [158], and inhibition of glucose transport and glucose phosphorylation [159]. The effects of leptin treatment on muscle may be partially attenuated by synthetic blockade of PI3-kinase activity [155], and leptin has profound effects on tyrosine phosphorylation of IRS-1, with no associated effect on insulin receptor phosphorylation [160]. Furthermore, a leptin-mediated recruitment of PI3-kinase to IRS-2 has been shown [161]. However, the model used in this study was hepatic cells and the activity at PI3-kinase did not translate to increased tyrosine phosphorylation of IRS-2 [161].

Leptin directly stimulates fatty acid oxidation in muscle by activating AMPK, an enzyme that phosphorylates and subsequently inactivates ACC [146]. Thus a direct mechanism for

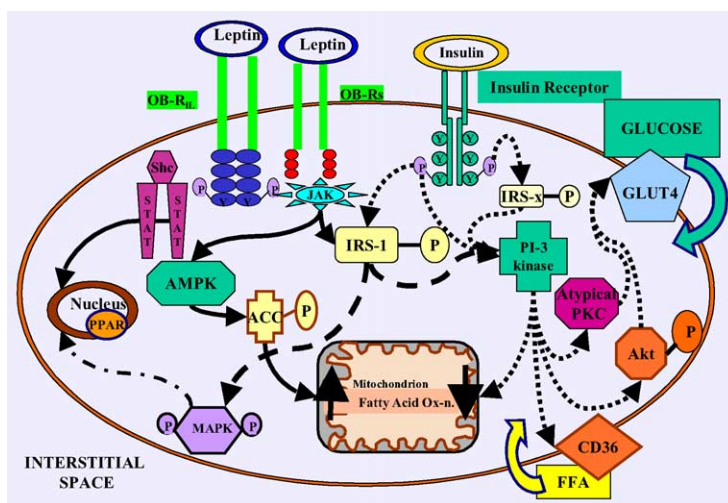


Fig. 4. This figure shows the pathways in muscle that may be unique to leptin binding/activation (solid line), those that may be unique to insulin binding/activation (short dots), and those that appear to be activated following binding/activation of either (long dashes). It must also be stated that much of the cascade down-stream of the insulin receptor may also be activated by insulin-like growth factors. It is not known whether any of the members of these cascades is limiting, although it is unlikely that activation by either the leptin or insulin axis causes recruitment of the entire cascade.

the action of leptin on fatty acid oxidation consistent with the work of Muoio et al. [69,155] has now been defined. Sweeney et al. [162] reported that leptin pretreatment in L-6 rat skeletal muscle cells reduced insulin stimulated phosphorylation of p35 MAP-K and the cAMP response element binder, resulting in a decrease in insulin stimulated glucose uptake. However, there was no effect of leptin on the insulin-stimulated increase in the GLUT4 translocation to the plasma membrane, and no effect on the signals thought to participate in mediating GLUT4 translocation, including tyrosine phosphorylation of IRS-1 and IRS-2, PI3-K activity, and serine phosphorylation of Akt. Accordingly, the molecular mechanism of reduced glucose uptake as a consequence of leptin appears to be a result of decreased stimulation of MAP-K, and reduced activation (MAP-K-dependent increase in intrinsic activity) [163] of GLUT4 instead of translocation [162].

Fig. 4 shows the pathways in muscle that may be unique to leptin binding/activation, those that may be unique to insulin binding/activation, and those that appear to be activated following binding/activation of either. It must also be stated that much of the cascade down-stream of the insulin receptor may also be activated by insulin-like growth factors [164]. It is not known whether any of the members of these cascades is limiting, although it is unlikely that activation by either the leptin or insulin axis causes recruitment of the entire cascade. Therefore, knowledge of the level of expression together with the level of phosphorylation and activation is required to aid our understanding of the leptin–insulin interaction in muscle. Note that there are alternate pathways to each of these shared mechanisms. For example, leptin’s actions are mediated via both JAKs and STATs, and no intersection

of activation of STAT with the insulin axis has been described. Thus, for each pathway there appear to be both independent and intersecting mechanisms. Therefore, it is possible that under appropriate physiological conditions, that each axis may function with minimal interaction or alternatively with maximal interaction.

Three separate studies have shown that leptin did not change insulin-stimulated metabolism in isolated rodent muscle [68,165,166]. In agreement with this finding Ranganathan et al. [167] showed that leptin had no effect on basal or insulin stimulated glucose transport in cultured rat and human skeletal muscle cells. However, Burcelini et al. [168] found that intravenous administration of leptin to ob/ob mice (which are insulin-resistant) increased glucose turnover and stimulated glucose uptake in brown adipose tissue (BAT), brain and heart. No increase in glucose turnover was observed in skeletal muscle or WAT. In another study of normal mice [169], infusion of murine leptin led to an increase in glucose turnover and 2-deoxyglucose uptake into skeletal muscle and BAT, increased whole-body glucose turnover and increased glucose oxidation; despite no change in plasma insulin or glucose concentrations. Furthermore, the leptin-induced increase in glucose uptake into extensor digitorum longus and soleus muscles was attenuated by denervation. Taken together, it appears that the effects of acute leptin administration on glucose metabolism in muscle are likely to be mediated via the CNS. Unfortunately, a number of these studies did not or could not investigate any down-stream effects on glucose metabolism, such as effects on glycogenesis or glucose oxidation, as the form of tagged glucose used was non-metabolizable (2-deoxy- or 3-O-methyl-glucose [166,167]). In only three of these studies has the leptin–insulin interaction effects on both glucose and fatty acid oxidation been investigated. All of these studies have reported increased oxidation of fatty acids in response to leptin treatment [68,155,170].

Szanto and Kahn [71] reported that leptin pretreatment enhanced insulin-induced tyrosine phosphorylation and PI3-kinase binding to IRS-1, and inhibited tyrosine phosphorylation and binding to IRS-2 in a hepatic cell line. Leptin also induced serine phosphorylation of Akt, a mechanism that down-regulates signaling in this pathway. It also up-regulated glycogen synthase kinase 3 (GSK3), indicating a potential role in enhancement of hepatic glycogen synthesis or modulation of hepatic function by changing gene expression or altering lipid metabolism [71]. Aiston and Agius [72] reported that leptin stimulated glycogen storage in hepatocytes by inhibiting phosphorylase-a in an additive manner with insulin, leading them to suggest leptin supports the thrifty genotype hypothesis by glucose conservation in the late absorptive state. However we believe that this is a further manifestation of the leptin drive toward sparing glucose in favor of fatty acid oxidation [8].

Fehmann et al. [70] suggested that leptin inhibited insulin secretion from pancreatic beta cells, and hypothesized that leptin was part of an “adipo-insular axis.” In this mechanism, insulin and glucagon appear to be signals to the adipocyte, directing lipid storage or release, and leptin serves as a signal that insulin is no longer necessary to induce lipid storage by adipocytes. In this same vein, Muller et al. [54] reported that leptin may impair the effects of insulin action on adipocytes, altering glucose transport, glycogen synthetase, lipogenesis, and protein synthesis. However, in the absence of insulin, leptin did not reduce glucose uptake and lipogenesis, supporting the idea that in adipocytes, not muscle, the effects of leptin may act in concert with insulin, perhaps via an insulin-signaling pathway [54].

#### 4.10. *Specific studies on autocrine/paracrine interactions*

Most of what has been discovered about different biological pathways and cellular interactions has been through the use of cell culture. Cell culture provides a means of determining specific pathways and effects of various treatments in a relatively simple system. Based on cell culture studies, it has been found that there are many autocrine and paracrine interactions between cells, and there are multiple influences between cell types. Therefore, cell co-culture systems are being actively developed in an attempt to more accurately mimic physiological conditions and provide a basis for investigating paracrine signaling between adipose and muscle tissue. For example, co-culture systems have been developed with ovine satellite cells and 3T3-L1 cells [171,172], endothelial cells and adipocytes [173], and adipocytes and skeletal muscle cells [174]. All of the aforementioned systems have found differences between cell types grown alone and those grown in co-culture systems, indicating crosstalk between cell types. For instance, Hossner et al. [172] developed a co-culture system with ovine satellite cells and 3T3-L1 cells, where it was reported that satellite cell viability was reduced by co-culture, while IGFBP secretion was increased. Dodson et al. [175] provided a review on the development and applications of this co-culture system. Dietze et al. [174] established a co-culture system of human adipocytes and skeletal muscle cells. They reported that there was decreased amount of insulin-stimulated activation of Akt kinase in myogenic cells in co-culture with adipocytes as opposed to culture with skeletal muscle cells only, indicating cross talk between adipocytes and myocytes. As these technologies progress, interactions between myocytes and adipocytes may be better determined. Currently, there is not extensive literature on co-culture systems between muscle and adipose tissue. However, co-culture could provide a useful means of understanding cellular communication, specifically interactions between myocytes and adipocytes.

### 5. Conclusion and applications

Regulation of growth, development, and differentiation in adipose tissue and skeletal muscle is controlled by a variety of endocrine, paracrine, and autocrine interactions. Research has begun to clarify the effects of some of these factors on adipose tissue and skeletal muscle. The signaling interaction between myogenic cells and adipocytes has been implicated as playing a significant role in the rate and extent of myogenesis, adipogenesis, and lipogenesis/lipolysis. These interactions have important implications in terms of our ability to ultimately influence relative fat and lean deposition and the efficiency of energy utilization in the complex processes of growth and development. Important factors that are involved in the regulation of these processes include glucocorticoids, insulin-like growth factors, various cytokines, insulin, adiponectin, and leptin. Interactions between these axes, their influence on fat and lean growth, and their effects on energy partitioning have also been implicated as important in the development of obesity and insulin resistance. Further studies are required to better understand and clarify the interactions among adipose tissue and muscle cells. As research progresses, pathways will become better understood, ultimately leading to optimized management of fat and lean growth in domestic livestock species.

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