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Key Proteins at the Interface of Bioenergetics and Mitochondrial Function

[1]W.A. Baldassini[1], [1]B.D. Dauria, [2]J.J. Ramsey, [3]R.H. Branco,
[3]S.F.M. Bonilha and [1]D.P.D. Lanna
[1]Department of Animal Science, "Luiz de Queiroz" College of Agriculture,
University of São Paulo (ESALQ-USP), Piracicaba, Sao Paulo, Brazil
[2]Department of Veterinary Medicine, Molecular Biosciences,
University of California Davis (UC Davis), Davis, California, United States
[3]Institute of Animal Science and Pastures-Instituto de Zootecnia (IZ),
Centro APTA Bovinos de Corte, Sertaozinho, Sao Paulo, Brazil

Abstract: The Shc molecules and the mitochondrial Uncoupling Proteins (UCP) have been proposed to play an important role in Energy Expenditure (EE) and cellular metabolism. We discuss the results of published studies regarding the influence of Shc proteins and UCP on energy metabolism of mice and beef cattle. Additionally, we review the possible association between mitochondrial function from animals classified according to Feed Efficiency (FE). Several studies have been conducted to investigate the role of Shc proteins play in aging and control of Reactive Oxygen Species (ROS) production. Studies have investigated the impact of low Shc levels (ShcKO) in preventing oxidative stress, apoptosis and hyperglycemia. In general, ShcKO is associated with changes in mitochondrial function and EE and protection of tissues against oxidative stress. However, little is known about the role of Shc proteins on energy metabolism in animals fed a high fat diet. In mitochondria, UCP activity provides adaptive thermogenesis, carbon flux maintenance and also protection of cell membranes against oxidative stress caused by ROS. In mitochondrial metabolism, UCP activity (uncoupling) is a paradigm in the context of FE. It may represent a cellular inefficiency but also a reduction in oxidative stress by attenuating mitochondrial ROS production. Thus, studies suggest that mitochondria from less FE animals have greater protein oxidation due to greater basal mitochondrial ROS generation. This increased ROS production could oxidize proteins, causing impaired protein synthesis. However, additional studies are needed to understand the physiological significance of these changes in mitochondrial function and energy metabolism and therefore, the impacts of these molecular mechanisms on animal performance and FE. Changes of very small magnitude in either mitochondrial function or enzyme activities could greatly alter energy metabolism and cause the changes in FE observed *in vivo*. Most of current biochemical studies are unable to detect the magnitude of the changes.

Key words: Energy expenditure, feed efficiency, metabolism, oxidative stress, Shc proteins, uncoupling

INTRODUCTION

There are three isoforms at the mammalian Shc locus, the highly expressed isoforms p46Shc and p52Shc and the minor p66Shc. The Shc molecule was initially described as a longevity protein, however, it has recently been shown that lifespan is not increased in mice with low levels of Shc proteins (Ramsey *et al*., 2013). Thus, Shc proteins do not alter lifespan but they do influence stress response (Migliaccio *et al*., 1999). However, cells derived from these mice exhibit lower levels of ROS (Nemoto *et al*., 2006). Although, several studies considered the depletion of p66Shc isoform as the main driver for changes in metabolism (Camici *et al*., 2007; Natalicchio *et al*., 2009), the p52Shc and p46Shc depletion in cells from ShcKO mice are the more likely candidates for alterations in insulin sensitivity and mitochondrial activation. Thus, decreases in the major Shc isoforms p52/p46 appear to drive both animal adiposity and insulin sensitivity phenotypes and also alterations in mitochondrial function (Tomilov *et al*., 2014).

Higher ROS generation may occur in tissues from Wild-Type (WT) compared to animals with low Shc protein levels. During mitochondrial respiration, electrons are extracted from substrates and transferred to molecular oxygen through successive redox reactions that are catalyzed by enzymatic complexes (termed Complexes 1-4). In the final step of this Electron Transfer Chain (ETC),

Corresponding Author: Welder A. Baldassini, Sao Paulo State University, FMVZ UNESP, Botucatu, Sao Paulo, Brazil

Table 1: Studies with different animal species regarding the role of Shc proteins on celullar metabolism and regulation of reactive oxygen species (ROS)

Reference	Species	Hypothesis	Results
Camici *et al.* (2007)	Mice	Shc proteins are involved with hyperglycemia and diabetes mellitus.	Deletion of p66Shc protects against hyperglycemia-induced and ROS-dependent endothelial dysfunction
Natalicchio *et al.* (2009)¥	Mice	Shc proteins are involved with glucose transport in skeletal muscle	p66Shc regulate the glucose transport system in skeletal muscle by controlling, via MAP kinase, the integrity of the actin cytoskeleton and by modulating cellular expression of GLUT1 and GLUT3 transporter proteins
Betts *et al.* (2014)	Cattle	Shc proteins regulates the oxidative stress response in bovine early embryo development	p66Shc knockdown (ShcKO) embryos exhibited reduced intracellular ROS and DNA damage (were stress resistant), exhibiting reduced oxidative stress and apoptosis
Perrini *et al.* (2015)	Human	Shc proteins mediates oxidative stress-related injury in liver cells and alcoholic steatohepatitis	Increased hepatocyte p66Shc protein levels may enhance susceptibility to DNA damage by oxidative stress by promoting ROS synthesis and repressing antioxidant pathways

¥MAP kinase = Mitogen Activated Protein Kinase; GLUT1 = Glucose Transporter 1; GLUT3 = Glucose Transporter 3

Table 2: Studies regarding the influence of Shc proteins on mitochondrial function and energy metabolism of mice

Reference	Hypothesis	Results
Nemoto *et al.* (2006)	Shc proteins are localized in mitochondria and regulate energy expenditure	The p66Shc is localized within the mitochondria and functions as a regulator of mitochondrial metabolism as well as regulate the partition of ATP generation in the cell
Orsini *et al.* (2006)	Shc proteins are regulated by transcription factors, protein stabilization and post-translational modifications	p66Shc activity is finely regulated both by cytosolic signals and by an intrinsic mitochondrial control, whereby changes in energetic status result in altered release of p66Shc
Trinei *et al.* (2006)	Shc proteins are involved in the regulation of mitochondrial DNA (mtDNA) copy number	p66Shc is part of the signaling pathway that regulates mtDNA replication both *in vitro* and *in vivo*
Hagopian *et al.* (2012)	Shc proteins influence enzymes involved in β-oxidation	Shc knockout mice showed increased liver and muscle β-oxidation enzyme activities in response to fasting and induce chronic increases in the activity of liver ketogenic enzymes
Stern *et al.* (2012b)	Shc proteins regulate whole body energy metabolism at 22°C and at 12°C (acute cold exposure).	Shc knockout mice demonstrate a slightly lighter total body mass and fat-free mass and therefore, energy expenditure was decreased in Shc knockout compared to wild-type animals (kJ/mouse or adjusted for body weight)
Bellisario *et al.* (2014)	Shc knockout mice are protected from the metabolic stressful induced by High Fat Diet (HFD)	Shc knockout mice showed greater resistance toward glucose challenge (by HFD) compared to wild-type and this Shc deletion results in insulin desensitization, acting as a protective factor 1

cytochrome c oxidase (Complex 4) ensures the reduction of molecular oxygen to water without the formation of oxygen radicals. However, partial reduction of oxygen with the generation of ROS can occur in sites other than Complex 4 with the mitochondrial ETC (Orsini *et al.*, 2006).

In mitochondria, ROS originated from the respiration process are inducers of oxidative damage and tissue dysfunction. Thus, when produced in excess, ROS affect many cellular processes such as energetic metabolism, signal transduction, gene expression, cell cycle and apoptosis (Orsini *et al.*, 2006). It is important to note that ROS may be produced in a controlled way through specialized enzymes and take part in regulating cellular processes (Bartosz, 2009). Several studies in mice, humans and cattle have been conducted to investigate the role Shc proteins play in cellular metabolism and oxidative damage (Table 1). In particular, these studies evaluated the impact of decreased Shc protein levels on preventing oxidative stress, apoptosis and hyperglycemia. It has been known that Shc proteins play a role in insulin signaling (Sasaoka and Kobayashi, 2000; Ravichandran, 2001) but relatively little has been known about the overall impact of these proteins on energy metabolism. The first evidence that Shc proteins may have an import influence on energy metabolism came from the observation that body weight and composition are altered in Shc knockout (ShcKO) mice. It was shown that body weight is decreased in ShcKO mice, despite the fact that their energy intake is not different than WT animals (Berniakovich *et al.*, 2008). This decrease in body weight is due to the fact that the weights of all fat pads are lower in ShcKO compared to WT mice (Berniakovich *et al.*, 2008; Tomilov *et al.*, 2011) (Table 2).

Similarly, it has been reported that ShcKO mice are resistant to weight gain on a HFD (Berniakovich *et al.*, 2008) and ShcKO in leptin-deficient ob/ob mice decreases weight gain in these obese animals (Ranieri *et al.*, 2010). Insulin sensitivity and glucose tolerance were increased in ShcKO mice (Ranieri *et al.*, 2010; Tomilov *et al.*, 2011) and this change in insulin sensitivity would be expected to influence energy metabolism in the fed state. However, results indicate that there are substantial changes in energy metabolism in ShcKO mice in the fasted state (Hagopian *et al.*, 2015). In particular, enzyme activity measurements in skeletal muscle and liver indicate a shift in metabolism towards increased capacity for fatty acid oxidation, ketogenesis, ketone body catabolism, gluconeogenesis and amino acid catabolism while

capacity for glycolysis is decreased in ShcKO compared to WT mice under fasting conditions (Clapham *et al*., 2000). These changes in enzyme activities mirror those observed in animals under aloric estriction CR) onditions (Hagopian *et al*., 2003; Stern *et al*., 2012a, b).

Additional studies with mouse models (ShcKO versus WT mice) were reported in the literature to investigate the role of Shc proteins in mitochondrial function and energy metabolism (Table 2). In general, the approaches associate decreased Shc levels with changes in the mitochondrial generation of ATP, regulation of mitochondrial DNA replication, energy expenditure and protection against oxidative stress in main body tissues following consumption of a HFD. It is important to note that the p66Shc knockout mice used by investigators have recently been shown to have low levels of all Shc isoforms in many tissues (Tomilov *et al*., 2014; Hagopian *et al*., 2015). Thus, it is difficult to determine if observed changes are truly due to p66Shc or due to one of the other Shc isoforms.

MATERIALS AND METHODS

Shc proteins and body composition: Studies have shown no differences in energy expenditure (adjusted for body weight or lean body mass) or Respiratory Quotient (RQ) between ShcKO and WT when allowed *ad libitum* access to food (Stern *et al*., 2012b). Similarly, there were no difference in energy expenditure and RQ between *ad libitum* fed groups of mice when exposed to an ambient temperature of 12°C for 24 h (Stern *et al*., 2012a, b). However, energy expenditure was decreased ($p<0.05$) in the ShcKO versus WT mice immediately following the initiation of CR. Mice were sacrificed following completion of the calorimetry measurements and body composition was determined including organ and fat pad weights. Very clear decreases in all fat pad weights were noticed in 1 year old ShcKO mice and it was also noticed that small but significant ($p<0.05$), decreases in epididymal, perirenal and subcutaneous fat pads were already present at 3 months of age (Fig. 1).

Shc proteins and enzymes activities of energy metabolism: Significant differences ($p<0.05$) were observed between the two groups of mice (WT vs. ShcKO) for enzyme activities in the major pathways of intermediary metabolism as follow: Glycolytic metabolites were decreased in ShcKO versus WT mice ($p<0.05$) corresponding with a decrease in glycolytic enzyme activity, Ketone body levels were increased in ShcKO versus WT mice, corresponding with increased ketone body synthesis. Metabolite levels are consistent

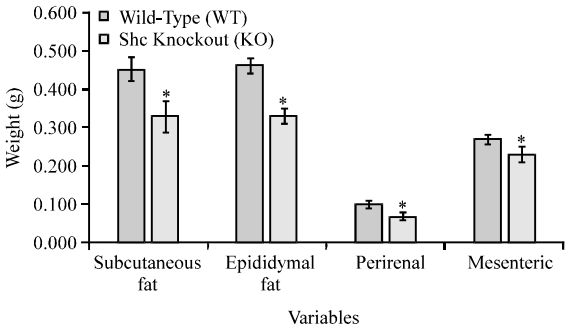

Fig. 1: Fat pad weights in Knockout (KO) and Wild-Type (WT) mice at 3 months of age and ad libitum fed on chow diet. *$p<0.05$ and ¥ $P<0.10$ between KO and WT mice (Hagopian *et al*., 2012)

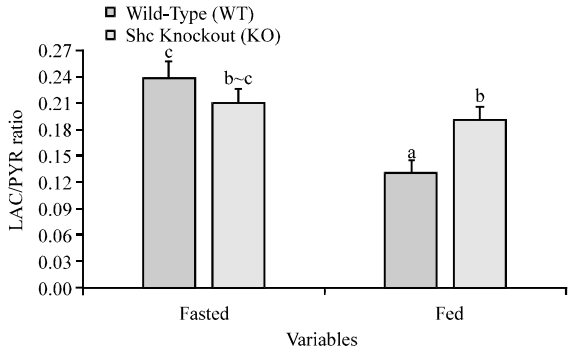

Fig. 2: Ratios of muscle lactate/pyruvate as indicators of redox state in the cytosol in Shc Knockout (KO) and Wild-Type (WT) mice. Animals were compared within a genotype, fasted versus fed, across genotype, fasted versus fasted and fed versus fed (Hagopian *et al*., 2015)

with a shift in substrate metabolism towards increased fatty acid and decreased glucose oxidation during fasting in ShcKO mice (Tomilov *et al*., 2011, 2016; Hagopian *et al*., 2012). Levels of lactate and pyruvate were also measured and the ratio of lactate to pyruvate was taken to indicate the redox state of the cytosol (Hagopian *et al*., 2015). These results indicate that cytosolic redox state was significantly altered in fasted ShcKO versus WT mice (Fig. 2).

RESULTS AND DISCUSSION

β(beta)-oxidation and ketone body metabolism: The activities of the β(beta)-oxidation enzymes acyl-CoA dehydrogenase, hydroxyacyl-CoA dehydrogenase and ketoacyl-CoA thiolase were measured in skeletal muscle

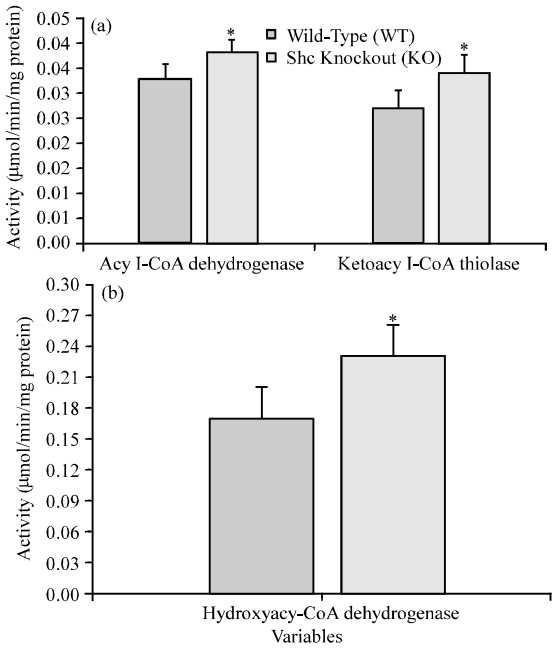

Fig. 3: Activities of β(beta)-oxidation enzymes in skeletal muscles of Shc Knockout (KO) and Wild-Type (WT) mice. *p<0.05 between KO and WT mice (Hagopian *et al*., 2012)

from fed and fasted ShcKO and WT mice (Hagopian *et al*., 2012). In the fed state, there were no differences (p>0.05) in the activities of any of these enzymes between the groups of mice. However, the activities of all of the β(beta)-oxidation enzymes were increased (p<0.05) in ShcKO versus WT mice following an overnight (16 h) fast (Fig. 3).

The activities of enzymes involved in ketone body synthesis and catabolism were also measured in skeletal muscle and liver (Hagopian *et al*., 2012). In both liver and muscle, the activities of β(beta)-hydroxybutyrate dehydrogenase and acetoacetyl-CoA thiolase were increased (p<0.05) in both the fed and fasted states in the ShcKO compared to WT mice. Overall, these results indicate that skeletal muscle from the ShcKO animals has an increased capacity for oxidizing both fatty acids and ketone bodies (Tomilov *et al*., 2011, 2016). We recently used ShcKO mice to determine the influence of Shc proteins on the metabolic response to acute feeding of a HFD (Baldassini *et al*., 2017). In this study, we report higher energy expenditure in ShcKO versus WT mice when consuming the HFD. Although, decreased levels of Shc proteins influenced the activity of some enzymes in response to high fat feeding such as increasing the activity of acyl-CoA dehydrogenase, it did not produce concerted changes in enzymes of glycolysis, citric acid cycle or the ETC.

Gluconeogenesis and glycolysis: The activities of the regulatory enzymes of gluconeogenesis (glucose-6-phos-phatase, fructose-1,6-bisphos-phatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase) were measured in liver from fed and fasted ShcKO and WT mice (Hagopian *et al*., 2016). There were no differences in enzyme activities between the groups in the fed state but in the fasted stated, the activities of phosphoenolpyruvate carboxylase and glucose-6-phosphatase were increased (p<0.05) in the ShcKO versus WT mice. These results are consistent with an increased capacity for gluconeogenesis in the ShcKO mice under fasting conditions (Hagopian *et al*., 2015).

In the same study, the activities of key regulatory enzymes of glycolysis (hexokinase, phosphofructokinase-1 and pyruvate kinase) were measured in skeletal muscle from fed and fasted mice (Hagopian *et al*., 2015). The activities of all three enzymes were decreased (p<0.05) in the ShcKO compared to WT animals under both fasting and fed conditions, although, the magnitude of these differences were greatest in the fasted mice (Fig. 4).

The citric acid cycle: Measures of enzyme activity indicated no overall changes in the citric acid cycle between ShcKO and WT mice under fasted or fed conditions (Hagopian *et al*., 2012). These results indicate that Shc proteins may influence the fuels used for energy but they do not produce a net change in overall capacity for energy metabolism in the mitochondria. Shc proteins may play an important role in energy metabolism and particularly, a decrease in Shc levels leads to an increased capacity for β(beta)-oxidation, ketone body metabolism, amino acid catabolism and gluconeogenesis under fasting conditions (Hagopian *et al*., 2012; Tomilov *et al*., 2016). Decreases in Shc proteins may play an important role in transitioning the animal from a fed to a fasted state. Thus, a decrease in Shc levels may help animals adapt to periods of CR or chronic consumption of low carbohydrate or HFD. However, little is known about the role of Shc proteins in energy metabolism in animals fed a HFD. More studies are needed to determine if Shc proteins play any role in changes in energy expenditure or enzymes activities of major metabolic pathways in response to a HFD.

The Uncoupling Proteins-UCP: The UCPs are localized in the inner membrane (Fig. 5) of mitochondria. UCPs are involved in different processes such as control of ATP synthesis, modulating ROS production and regulation of fatty acid metabolism (Echtay, 2007). These proteins may increase energy expenditure in tissues, generating great

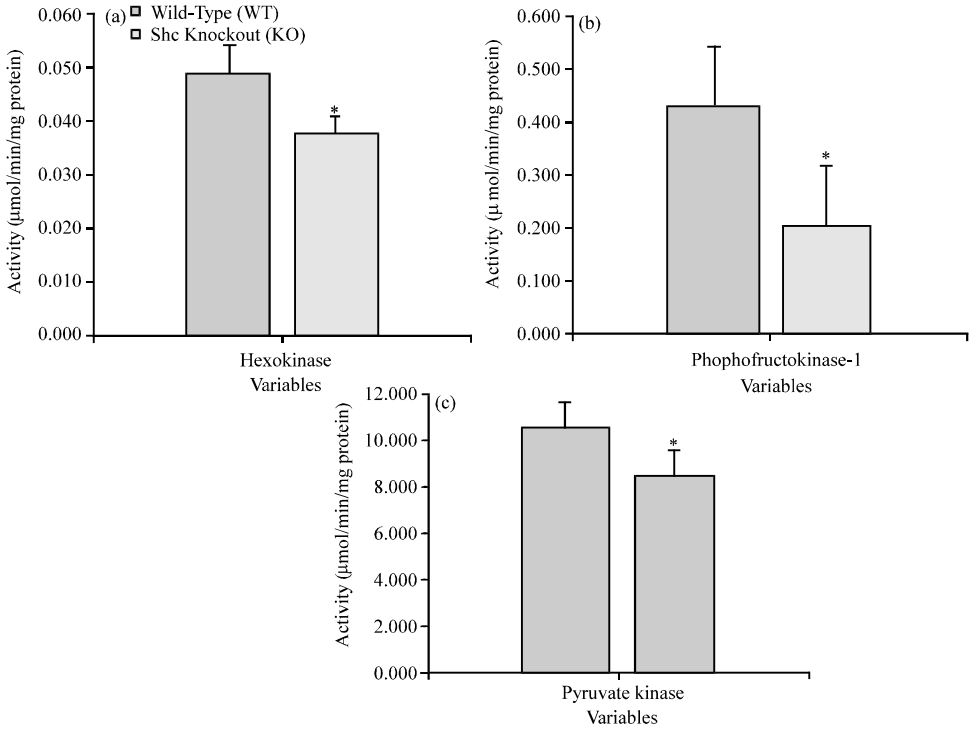

Fig. 4: Activities of glycolytic enzymes in skeletal muscles of fasted Shc Knockout (KO) and Wild-Type (WT) mice. *p<0.05 between KO and WT mice (Hagopian *et al*., 2015)

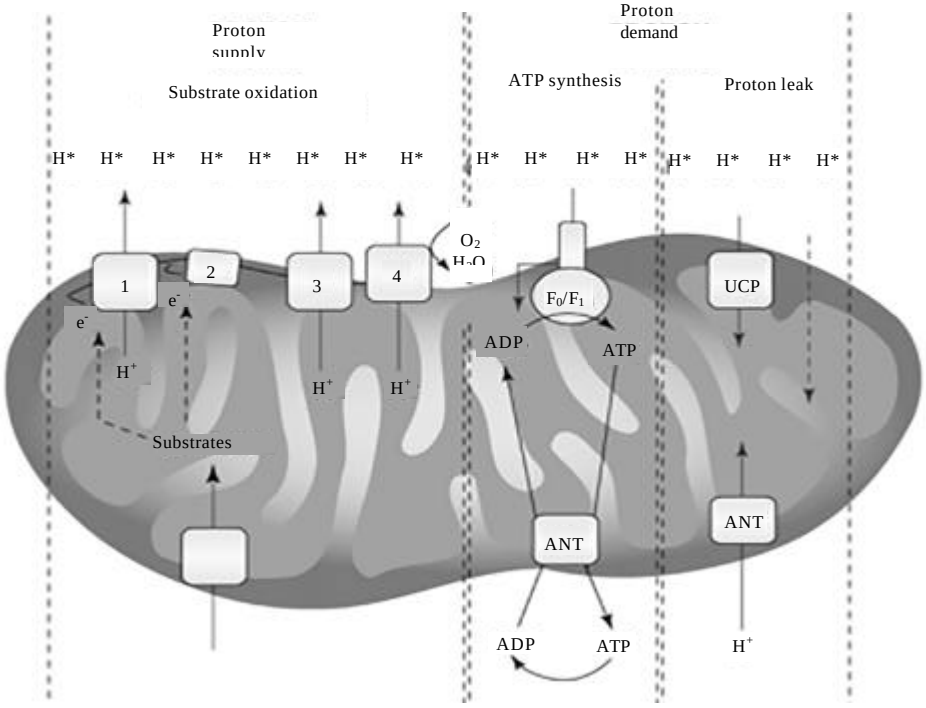

Fig. 5: Oxidative phosphorylation and proton leak pathways in mitochondria. Proton leak pathways can be mediated by Uncoupling Protein (UCP) or by Adenine de Nucleotide Translocase (ANT) (Azzu and Brand, 2010)

interest in the potential role they play in obesity, diabetes and energy metabolism as reviewed (Bouillaud *et al*., 2016).

It is important to understand how and why the uncoupling process occurred. The respiratory substrates are oxidized at mitochondrial respiratory complexes 1-4, leading to the ejection of protons (H^+) into the intermembrane space. This proton electrochemical gradient is consumed by the F_0/F_1 ATP synthase to produce ATP or by proton leak pathways which stimulate heat production without ATP synthesis (Azzu *et al*., 2010). This proton leak is the movement of protons through the inner membrane of mitochondria mediated by UCPs (main pathway), Adenine Nucleotide Translocase (ANT) and other carriers and porosities (Azzu and Brand 2010; Azzu *et al*., 2010). Proton leak represents an inefficiency that occurs because there is an incomplete coupling of oxidative phosphorylation. On the other hand, proton leak mediated by UCPs provide adaptive thermogenesis, carbon flux and protection of cell membranes against oxidative stress (Azzu *et al*., 2010; Divakaruni and Brand, 2011).

The UCP family: The first member of the UCP family, Brown adipose Tissue (BAT) Uncoupling Protein 1 (UCP1) was identified in 1976. Twenty years later, two closely related proteins, UCP2 and UCP3, were described in mammals (Bouillaud *et al*., 2016). In adipose tissue, adipocytes can be divided into white and brown cells. While white cells are specialized in storing chemical energy, brown adipocytes produce heat through a large amount of mitochondria-rich in UCP1 (Wu *et al*., 2013). UCP1 is known for its role in adaptive and noshivering thermogenesis (neonates and hibernation in animals). Studies have also investigated the role of UCP1 in the regulation of body weight (Azzu *et al*., 2010; Divakaruni and Brand 2011) and UCP1 appears to regulate the body weight of animals by activating the thermogenic program in adipose tissue, producing heat and burning off energy from oxidized substrates (Wu *et al*., 2013).

The UCP2 gene is widely present in tissues and in contrast to UCP1 it is expressed not only in BAT but also in White Adipose Tissue (WAT), skeletal muscle, heart, kidney, lung, spleen and others (Erlanson-Albertsson 2003). Whereas UCP2 is widely expressed in tissues, UCP3 is expressed almost exclusively in skeletal muscle and brown adipose tissue and to a lesser extent in the heart (Mailloux and Harper, 2011).

In humans, tremendous interest has been generated in targeting energy expenditure in order to provide treatments to obesity (Wu *et al*., 2013). This could be possible by increasing activation of brown adipocyte progenitors to induce BAT differentiation and increasing UCP1-mediated uncoupling. However, there is a need for an explicit description of UCP1 mechanism (Divakaruni and Brand, 2011) and only few studies with mice have try to describe this process. On the other hand, particularly in skeletal muscle, a study describe that increasing the uncoupling is an effective obesity treatment, since, over expression of UCP3 in mice causes fat-specific weight loss (Clapham *et al*., 2000) and additionally, a study demonstrated that deranged expression of UCP confers resistance to diet induced obesity (Li *et al*., 2000).

UCPs, mitochondrial function and feed efficiency: The role of UCPs in these physiological processes in the liver, muscle and adipose tissue may account for inter-animal variation in energy expenditures and heat production. In addition because mitochondria produce approximately 90% of the energy for the cell how efficiently this process is conducted has implications in animal growth and development as reviewed (Bottje and Carstens 2009, 2012). In this context, several studies focus on the association between mitochondrial function, UCP activity and FE of beef cattle (Kolath *et al*., 2014), poultry and livestock species (Bottje and Carstens, 2009; Grubbs *et al*., 2014). Additional studies and their main results were summarized (Table 3).

Transcription factors and other genes encoding mitochondrial proteins may be critical determinants of cellular function associated with the phenotypic expression of FE (Kolath *et al*., 2006a, b; Sherman *et al*., 2008). Moreover, the sequencing of cattle genome provides resources for accessing the genotype and phenotype relationships which can be used to improve FE of animals as reviewed (Kim and Seo, 2012). However, typical results relating to UCP mRNA levels (Fonseca *et al*., 2015) must be taken with extremely attention, since it was demonstrated that UCP mRNA does not produce heat (or it is related to energy expenditure) and UCP protein levels would be the more relevant parameter to measure (Nedergaard and Cannon 2013). The increase in total UCP1 amount, for example, correlates temporally with the increase in nonshivering thermogenesis whereas changes in UCP1 mRNA or specific UCP1 protein levels do not correlate (Nedergaard and Cannon, 2013).

Moreover, in FE studies the animals ranked for Residual Feed Intake (RFI) (Koch *et al*., 1963) have been

Table 3: Studies regarding the role of Uncoupling Proteins (UCP) and mitochondrial function on biological efficiency and animal performance

Reference	Animal	Hypothesis	Laboratory assay ¥	Tissue	Results
Ojano-Dirain *et al.* (2007	Broiler	Avian UCP (avUCP) gene expression in efficient versus inefficient birds	Quantitative real time PCR	Muscle	avUCP mRNA levelswere greater in breastmuscle of inferior feed efficiency broilers
Asano *et al.* (2013)	Beef cattle	UCP1 gene expression in adipose tissue of cattle fed with concentrate versus roughage diet	Immunohistochemical analysis +Quantitative real time PCR	Subcutaneous and visceral fat	UCP1 mRNA expression in the subcutaneous fat was higher in the concentrate diet group than in the roughage diet group
Grubbs *et al.* (2013)	Pigs	Mitochondrial proteome of efficient versus inefficient in animals selected for Residual Feed Intake (RFI)	2D DIGE+Mass spectrometry	Muscle	Selection for RFI alters the mitochondria proteome in the low RFI line
Casal *et al.* (2014)	Beef cows	Gene expression of Electron Transport Chain (ETC) proteins in pure and crossbred animals grazing different forage allowances of native pastures (high or low)	Quantitative real time PCR	Small intestine and liver	Greater expression of ETC protein in the small intestine of high than low cows, and also in crossbreed than pure cows
Fonseca *et al.* (2015)	Beef cattle	UCPs gene expression in efficient versus inefficient RFI phenotypes	Quantitative real time PCR	Liver and muscle	Liver UCP2 mRNA expression levels is greater in low than high RFI cattle

¥PCR = Polymerase Chain Reaction; 2D DIGE = Two-Dimensional Difference Gel Electrophoresis

shown to have different compositions of body weight growth. More efficient animals (lower RFI) have leaner carcasses (Nascimento *et al.*, 2016) and less internal fat (Gomes *et al.*, 2012; Basarab *et al.*, 2013). Thus, changes in metabolism and intake may not only be related to changes in energy expenditure but also to changes in body tissue composition. Taken together these results suggest that RFI ranking could introduce a bias as it deviates from energetic efficiency rankings.

Mitochondrial function on biological efficiency of energy metabolism: A study by Herd *et al.* (2004) suggested that approximately 67% of variation among animals that are efficient and those that are inefficient relate to basal metabolic rate, cellular maintenance requirements and energy lost as heat. In their study, for animals classified according to RFI, one-third of the biological variation of growing cattle could be explained by interanimal differences in digestion, heat increment, the composition of growth and activity and posited that the remaining two-thirds was linked to interanimal variation in energy expenditure including the mitochondrial efficiency and metabolism. However, these results should be taken with caution, since, it is not clear how these values were estimated by the resesarchers in order to explain the variation in FE and RFI.

In the physiological processes described by Herd *et al.* (2004), it has been estimated that mitochondrial proton leak, Na+/K+ ATPase and protein turnover each contribute approximately 20% to the total interanimal variation in basal energy expenditures (Bottje and Carstens 2009). Reaction in which energy expenditure is not directly controlled through hydrolysis of high-energy phosphatic bounds (proton leakage, protein turnover) could contribute significantly to variation in efficiency (Herd and Arthur, 2009). However, these reactions remain difficult to quantify even in experimental conditions.

The paradigm in mitochondrial metabolism is that uncoupling by UCP may represents a cellular inefficiency but also reduces oxidative stress by attenuating mitochondrial ROS production. A study reported that proton leak in isolated muscle mitochondria obtained from superior FE phenotype broilers treated with chemical inhibitors of cellular respiration process was consistently less than that observed in mitochondria isolated from broilers with inferior FE (Bottje and Kong, 2013). Thus, the researchers suggest differences in membrane characteristics that affect proton conductance in broiler muscle mitochondria which could contribute to higher mitochondrial ROS associated with the phenotypic expression of inferior FE (Bottje and Carstens 2009, 2012). Additionally, in birds, the selection for decreased breast mitochondrial content might be expected to increase FE (Hudson *et al.*, 2017).

Furthermore, a study showed that cattle with low RFI had a greater coupling of oxidative phosphorylation in skeletal muscle mitochondria than cattle with high RFI (Kolath *et al.*, 2006a, b). However, the physiological significance of these association including mitochondrial function, proton leak, UCPs activity (uncoupling) and FE remains to be determined. Few studies have been able to confirm these results across species.

Limitation in reviewed studies

Shc proteins: With ShcKO mice model used in the studies it is not possible to determine which specific Shc isoform is responsible for observed changes in enzyme activities

or energy expenditure. Additional models that allow controlled expression of specific Shc isoforms are needed to further dissect the contribution of the individual isoforms to changes in metabolism following consumption of a HFD. In addition, the extremely low Shc expression may alter the whole system to a dysfunctional state which is possible in knockout models.

All of this is complicated by the fact that much but importantly not all are phenomena collected from intact animals, tissues or cells. Additionally, some studies that report specific changes in enzyme activities or metabolite levels are small magnitude and their overall significance is difficult to be useful.

Uncoupling proteins: Currently, the studies were in general unable to demonstrate differences in UCP abundance on a per cell basis in groups of animals inferior or superior for feed efficiency. The results of published studies with cattle suggest no apparent differences in mitochondria abundance or in electron transport chain and oxidative phosphorylation in liver, muscle and adipose tissue from these groups of animals differing in efficiency. However, additional studies measuring UCP genes and protein amount are needed. Changes of very small magnitude in either mitochondrial function or enzyme activities could greatly alter energy metabolism and cause the changes in feed efficiency observed *in vivo*. Most of current biochemical studies are unable to detect the magnitude of the changes in RFI observed in beef cattle. These small energy expenditure changes are of enormous economic significance.

CONCLUSION

In the context of energy metabolism and mitochondrial function, Shc proteins should be considered as potential molecular markers in genetic selection due to its association with weight gain and body composition of animals. It could be proposed to be used as a means to manipulate gain, body composition and efficiency. However, the reviewed studies outlines very specific observations when Shc is ablated and the mechanism that overall change animal energy metabolism and body composition remains obscure.

Although, proton leak and the total abundance of mitochondria are key to any estimate of feed efficiency traits, it remains very difficult to determine these in organs or animals. The variation in efficiency in farming animals is extremely low (2-5% being of great economic importance). Studies conducted to observe differences of these magnitudes in proton leak, mitochondrial abundance, energy coupling or gene expression are not common. The assays were in general unable to demonstrate differences in UCP abundance on a per cell basis in groups of animals inferior or superior FE. Thus, additional studies measuring protein amount or enzyme activity would be needed.

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