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Variation of carnitine concentrations in Angus beef

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by

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter I

Chapter II

LIST OF TABLES

Chapter I

Chapter II

LIST OF ABBREVIATIONS

FC: free carnitine

AC: acylated carnitine

TC: total carnitine

HCW: hot carcass weight

Fat12: fat at rib 12

REA12: rib eye area at rib 12

YG: yield grade

MS: marbling score

WBS: Warner-Bratzler Shear force

KPH: internal fat (perirenal, pericardial, and pelvic fat;

CPT I: palmitoyltransferase I

CPT II: palmitoyltransferase II

CT: carnitine translocase

CACT: carnitine-acylcarnitine translocase

CoA: coenzyme A

ACoAS: acyl-CoA synthetase

LCFA: long-chain fatty acids

CAT: carnitine acetyltransferase

TML: trimethyllysine

TMLD: trimethyllysine deoxygenase

HTML: 3-hydroxyl trimethyllysine

TMABA: 4- Trimethylaminobutyraldehyde

TMABA-DH: 4-Trimethylaminobutanal dehydrogenase

BB: butyrobetaine

BBD: butyrobetaine dioxygenase

IA: Iowa

CA: California

CHAPTER I: GENERAL INTRODUCTION

Carnitine (3-hydroxy-4-trimethylaminobutyrate) is prevails in mammalian tissues and plasma and within the skeletal and cardiac muscles. Carnitine exists in two isomer forms: D-carnitine (inactive form) and L-Carnitine (active form). Carnitine is made available to the body by two independent pathways: an endogenous biosynthesis from methionine and lysine and from dietary sources, such as meat and dairy products. L-Carnitine in humans and animals tissues is present in two forms: free carnitine (FC) and acylated carnitine (AC) (Shruti and Stephen, 2010; Figure 1). Normally, FC constitutes approximately 80% of TC (Kenichiro et al., 2004).

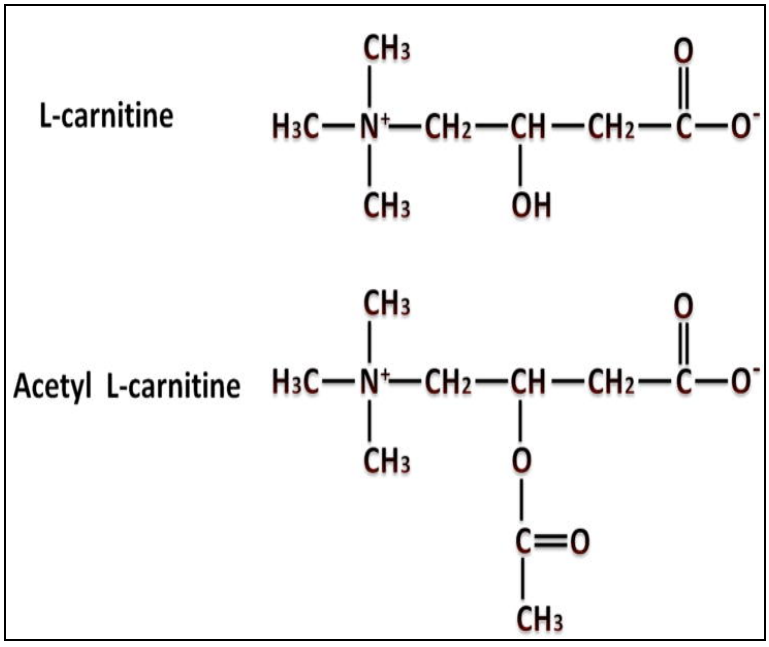

Figure 1. Chemical structures of free L-Carnitine and acetylated carnitine. Adopted from Shruti and Stephen (2010).

Function of Carnitine

The primary function of L-Carnitine is to transport and enhance entry of long-chain fatty acids (LCFA) into the mitochondrial matrix for ß-oxidation (Figure 2) (Reda et al., 2003). Additionally, L-Carnitine buffers excess production of acetyl-CoA in the mitochondria by producing AC with concomitant production of coenzyme A (CoA-SH), which plays a main role in various mitochondrial energy metabolic processes. It has been

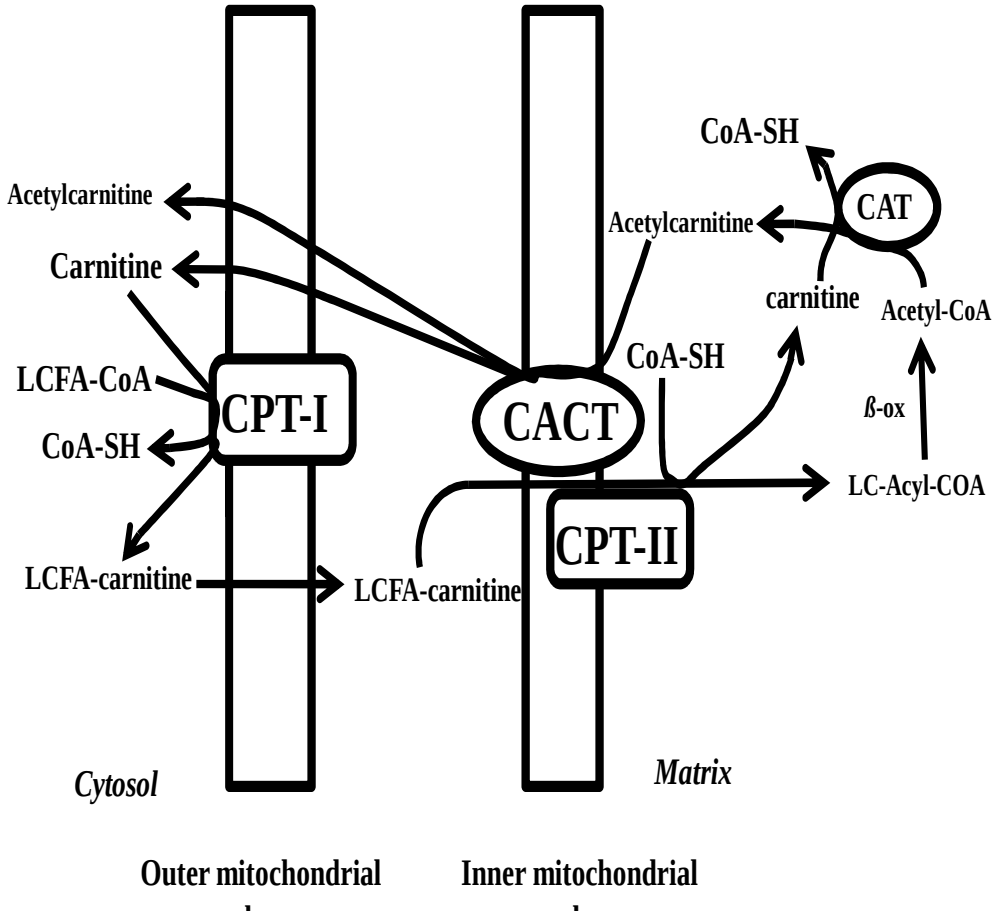

Figure 2. Function of carnitine in the transport and oxidation of mitochondrial long-chain fatty acyl and regulation of mitochondrial acyl-CoA/CoA ratio. Carnitine palmitoyltransferase I (CPT I), coenzyme CoA (CoA), carnitine-acylcarnitine translocase (CACT), long-chain fatty acids (LCFA), carnitine palmitoyltransferase II (CPT II), carnitine acetyltransferase (CAT).

shown that AC plays two vital metabolic functions. First, it transports fatty acyl groups among various body organs. Second, it detoxifies excess acyl compounds produced from the metabolism of xenobiotic compounds, such as, valproyl- CoA (Lheureux et al., 2005). As was previously emphasized, the primary function of L-Carnitine is to facilitate *ß*-oxidation of esterified fatty acids in the mitochondrial matrix. An uptake disorder of L-Carnitine by skeletal or cardiac myocytes can induce myopathy that progresses to cardiac diseases (Lahjouji et al., 2001). Additionally, L-Carnitine transports very long-chain fatty acids to the peroxisomes to undergo ß-oxidation that generates long-chain fatty acids that subsequently become suitable substrates for the mitochondria (Wanders, 2000). Thus, carnitine is critical for fatty acids catabolism and energy homeostasis.

Acetyl-CoA generated by *β*-oxidation of fatty acids, is an important intermediate in glucose and fatty acid metabolism. To be oxidized to CO_2, acetyl-CoA is condensed with oxaloacetate to form citrate in a reaction catalyzed by citrate synthase in TCA cycle in skeletal muscle and other aerobic tissues (Wachter et al., 2002). Of importance, during accelerated lipolysis, carnitine converts excess acetyl-CoA into AC. The conversion of acetyl-CoA into AC buffers the toxic effect of elevated intracellular acetyl-CoA and regenerates the reduced form of CoA (Kenichiro et al., 2004). However, inadequate CoA concentration decreases production of acetyl-CoA and N-acetylglutamate. Subsequently, carbamoyl phosphate synthetase-1 will be inactivated inhibiting urea cycle and causing an accumulation of ammonia (Scaglia, 2001). Thus, sufficient L-Carnitine concentration is an important factor in the cellular energy homeostasis. The concentrations of L-Carnitine in beef and ram rump have been shown to be higher than that of chicken, fish, or pigs (Table 1) (Shamada et al., 2004). Report by Nelson and coworkers (1985) indicated that in bovine

Table 1. L-Carnitine and myoglobin concentrations of semitendinosus muscles from various animals

Species	Age (mo)	L- Carnitine umol/g tissue (n)	Myoglobin mg/g tissue (n)
Chicken			
Broiler	1.5	0.69 ± 0.27 (6)	1.37 ± 0.08(4)
White leg-horn	3–4	1.33 ± 0.6 (33)	1.58 ± 0.04(27)
Pigs			
Large White x Landrace x Duroc	9	1.09 ± 0.06 (2)	1.01 ± 0.68(2)
Beef cattle			
Angus x Hereford	32	3.47 ± 0.04 (2)	3.98 ± 0.04 (2)
Japanese Black	32	3.57 ± 0.20 (3)	4.09 ± 0.14 (3)
Holstein, Steer	12	1.86 ± 0.49 (4)	1.87 ± 0.12 (4)
Holstein, Steer	24	2.04 ± 0.15 (2)	2.87 ± 0.28 (2)
Dairy cattle			
Holstein	36	2.73 (1)	4.11 (1)
Holstein	60	2.97 (1)	6.57 (1)
Calf			
Holstein	1	1.67 ± 0.46 (3)	1.49 ± 0.45 (3)
Horse			
Thoroughbred	3	2.79 (1)	1.89 (1)
Hokkaido native horse	36	4.95 (1)	5.84 (1)
Deer			
Hokkaido native deer	> 36	4.57 ± 0.62 (8)	6.01 ± 2.44 (4)
Goat	Unknown	11.36 ± 0.71 (3)	6.35 ± 1.18 (3)

Note. Values are means ± SD of the numbers (*n*) of samples. Adopted from Shamada et al. (2004).

longissmus dorsi muscle, L-Carnitine concentration diminishes gradually during postmortem aging, suggesting that a long a storage period might decrease carnitine concentration in beef. Worth mentioning, tissue L-Carnitine is quantified by a method based on the enzymatic reaction that generates acetylcarnitine and CoA from carnitine. This reaction is catalyzed by acetyl-CoA and carnitine acetyltransferase (Marquis and Fritz, 1964; Xia and Folker, 1991).

Carnitine Biosynthesis

The liver, kidney, and brain are the main human organs that carry carnitine synthesis (Rigault et al., 2006). Synthesis of carnitine (Figure 3) involves condensation of methionine and lysine as precursors followed by five sequential enzymatic reactions that include some cofactors such as ionic iron and ascorbate (Vaz and Wanders, 2002). During carnitine synthesis, lysine presents the carbon backbone of carnitine (Tanphaichitr et al., 1973), the 4-*N*-methyl groups are donated by the methionine in reaction catalyzed by methyl transferases. Specific mammalian functional proteins are rich in N^6-trimethyl-lysine (**TML**) residues (Paik et al., 1971). Such proteins include myosin, actin, cytochrome c, calmodulin (calcium-binding protein), and histones (Morse et al., 1975). The methyl groups are donated to the lysine by s-adenosylmethionie in a reaction catalyzed by methyltransferse enzymes. Eventually, TML is released via hydrolysis (Dunn and Rettura, 1984) and utilized to for the synthesis of new carnitine molecules.

Initially, TML undergoes hydroxylation at the 3-position in a reaction catalyzed by TML dioxygenase (**TMLD**), which uses Fe^{2+} and vitamin c as cofactors to yield 3-hydroxy-TML (**HTM**L). Next, 4-trimethylaminobutyraldehyde (**TMABA**) and glycine are generated by an aldolytic cleavage of HTML in a reaction catalyzed by HTML aldolase that uses PLP as cofactor. Dehydrogenation of TMABA by TMABA dehydrogenase (**TMABA-DH**), which has specificity for NAD^+ and TMABA results in the production of 4-*N*-trimethylaminobutyrate (**butyrobetaine; BB**). Eventually, BB is hydroxylated at the 3-position by butyrobetaine dioxygenase (**BBD**) to produce carnitine. Worth mentioning, BBD, similar to TMLD, recruits ascorbate and Fe^{2+} as cofactors (Vaz and Wanders, 2002). Newly synthesized carnitine is excreted in the blood and distributed to tissues that lack BBD activity

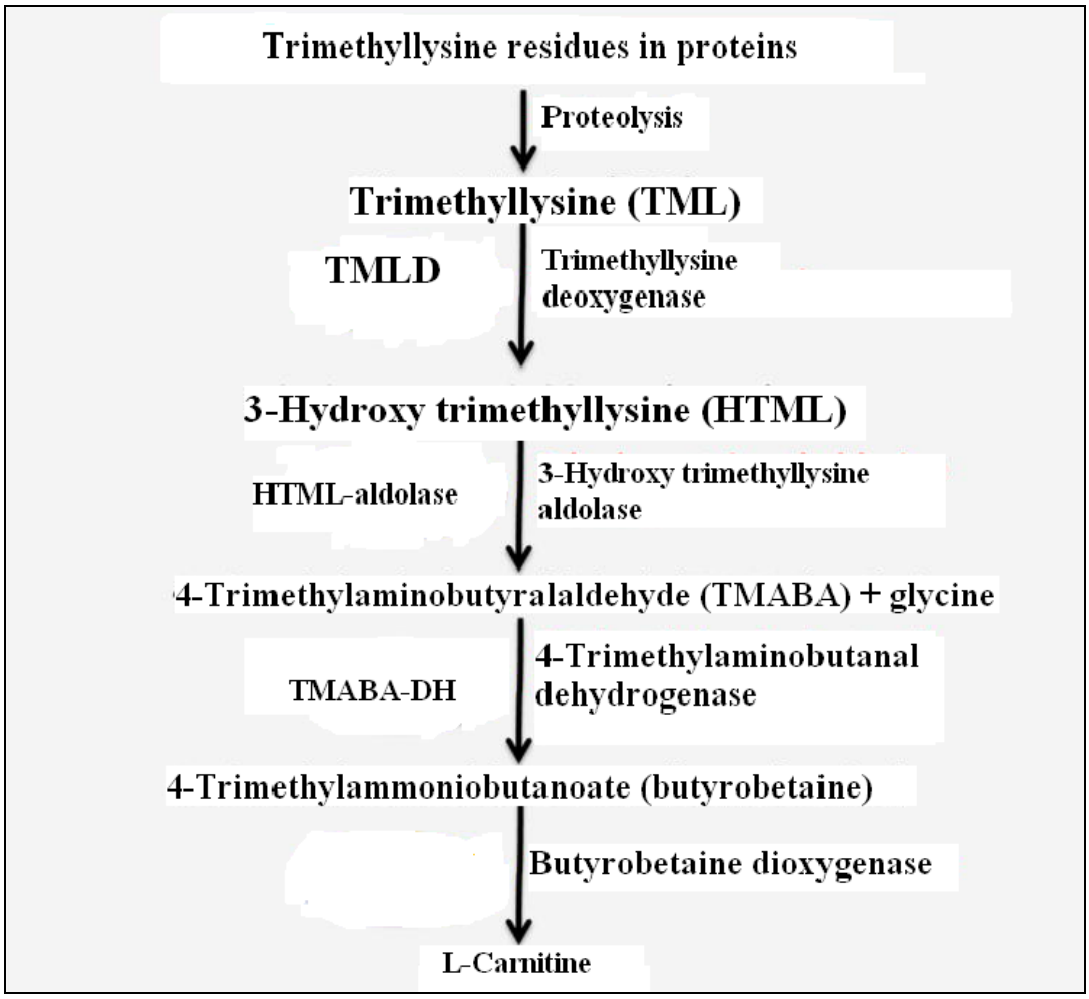

Figure 3. The carnitine biosynthesis pathway. Trimethyllysine (TML); trimethyllysine deoxygenase (TMLD); 3-hydroxyl trimethyllysine (HTML); 4-Trimethylaminobutyr-aldehyde (TMABA); 4-Trimethylaminobutanal dehydrogenase; (TMABA-DH); 4-N-trimethylaminobutyrate (butyrobetaine; BB) butyrobetaine dioxygenase (BBD); free carnitine (FC). Adapted from Shruti and Stephen (2010).

but depend on carnitine for their energy metabolism, such as the skeletal and cardiac muscles (Vaz and Wanders, 2002). Studies on carnitine homoeostasis in humans have unraveled that carnitine is endogenously synthesized in the liver, kidney, and brain. Non-renal and non-hepatic tissues do not contribute to carnitine synthesis and take up carnitine that is readily available in the circulation (Figure 4). Express Sequence Tag clones analyses indicated that carnitine might be synthesized endogenously in the testis and the lung (Rigault et al., 2006).

Additionally, sequencing of the genome of numerous organisms revealed the presence of homologous carnitine synthesis enzymes. Rebouche and Engel (1980b) investigated the tissue distribution of the enzymes involved in carnitine synthesis in humans.

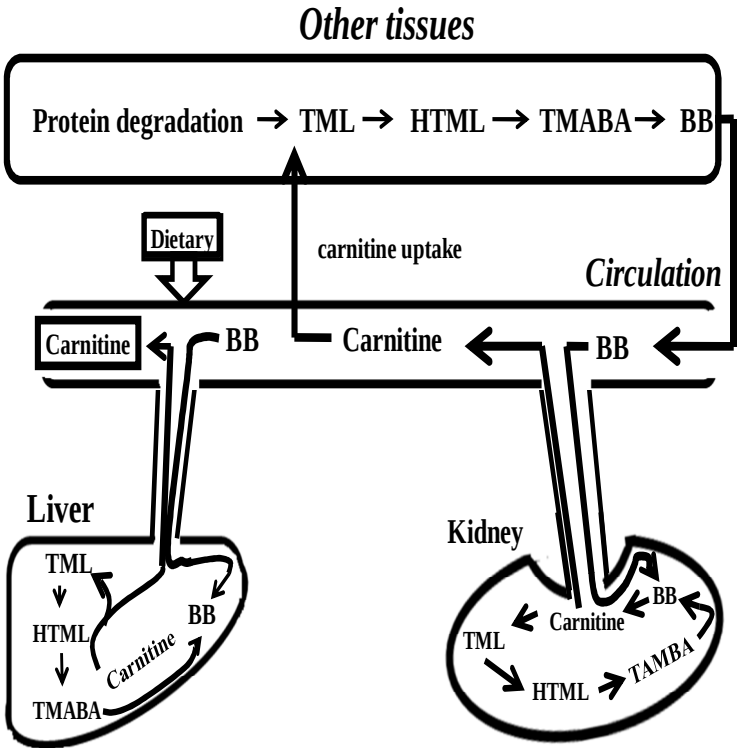

Figure 4. Schematic representation of carnitine homoeostasis. Carnitine is synthesized in the kidney, liver and brain (not shown). Trimethyllysine (TML); butyrobetaine (BB); butyrobetaine dioxygenase (BBD).

TMLD activity is detectable in the liver, heart, muscle, and brain; however, its activity is highest in renal tissues. In contrast, HTMLA is predominantly active in the liver compared with extrahepatic tissues. Similarly, the rate of TMABA oxidation is high in hepatic and renal tissues and low in brain and cardiac muscles. Thus, these results demonstrate that the enzymes necessary to convert TML into BB are widely distributed among different body tissues. The kidney, liver, and brain, however, are capable of hydroxylating BB into carnitine (Rebouche and Engel, 1980b). Also, TML is produced by the protein degradation.

TML can be directly converted to BB almost in all tissues. In contrast, only the liver, kidney, testis and brain are able to express BBD that catalyze the hydroxylation of BB to carnitine (Rebouche and Engel, 1980b). Tissues that lack BBD release BB in the circulation. Liver and kidney take up BB from circulation and convert it into carnitine. The kidney reabsorbs carnitine and BB efficiently, thereby decreasing their loss in urine. It was shown that BBD activity in kidney is about 3- to 16-fold greater than that of the liver (Englard, 1979). In comparison, BBD activity in the brain has been shown by Rebouche and Engel (1980b) to be only about 50% of its activity in hepatic tissues. In rodents and humans, hepatic BBD activity increases readily with age (Hahn, 1981; Englard, 1979). Activity of renal BBD, however, does not correlate with age and remains unaltered in newborn and adult humans (Olson and Rebouche, 1987). In contrast, the correlation of hepatic TMLD, HTML, or TMABA with age warrants investigation (Rebouche and Engel, 1980b).

Transport of Carnitine Metabolites

Until recently, the homeostasis of carnitine and of the metabolites associated with carnitine biosynthesis has not been completely clear. An earlier report by Zapel and

colleagues (1980) demonstrated that TML and HTML are absorbed by the intestine. To date, transporters associated with carnitine and its metabolites, such as TML and HTML, are poorly characterized. Similarly, the cellular concentrations of TML and HTML have not been determined. Because in rats the kidney is able to effectively reabsorb TML, it is likely that a system of carnitine transporters is present, at least in rodents. As cardiac and skeletal muscles, liver, and kidney are extremely dependent on the energy generated by ß-oxidation, it is crucial that these tissues have adequate carnitine. Carnitine concentration in tissues is generally 20- to 50-fold higher than that in plasma (Bremer et al., 1983). In humans, apart from kidney, liver, and brain that have the required enzymes to synthesize carnitine, most tissues take up plasma carnitine by sodium-dependent active transport. Kinetic studies of the plasmalemmal carnitine transporter by Rebouche and Engel (1982) have shown analogous similar K_m values of 2-60 µM for carnitine transport in skeletal muscle (Rebouche, 1977), fibroblasts (Tein et al., 1990), heart (Bahl et al.,1981), and placenta (Prasad et al., 1996), indicating a common carnitine transporter. This same carnitine transporter seems to carry renal reabsorption of carnitine (Rebouche and Engel, 1980a). Carnitine transporters that actively transport carnitine have been identified. cDNA sequencing study by Wu et al. (1998) has identified the organic cation transporter 2 (OCTN2). This *OCTN2* gene expression is robustly inhibited by BB and acetylcarnitine, indicating a role for OCTN2 in carnitine transport (Tamai et al., 1998). Later, it was shown that OCTN2 localizes to the apical membrane of renal tubular epithelial cells and facilitates carnitine reabsorption (Tamai et al., 2001).

Carnitine Degradation and Excretion

Both FC and AC are chiefly excreted through urine (Brass and Hoppel, 1978). In rats, about 20 pmol of carnitine are excreted daily per gram of body weight (Tasi et al., 1975). This amount represents about 5% of the total body pool suggesting sluggish homeostasis in skeletal muscle (Brooks and Mcintosh, 1975). Under physiological conditions, renal clearance of carnitine in rats is less than 50 milliliters daily. The glomerular filtration rate is ~17 milliliter/g body weight daily (Bauman et al., 1970). Thus, most of the carnitine in the glomerular filtrate is reabsorbed by the kidney. In contrast, in fasting rats, both the plasma concentration and the renal excretion decrease initially followed by an increase above the initial values after a 3- to 4-day fast. Additionally, carnitine is as well excreted through the mammary gland into milk raising milk carnitine concentration to about 0.3 mM (Erfle and Sauer, 1974). Feeding high amounts of carnitine to and rodents substantially increased urinary excretion of trimethylamine, trimethylamineoxide, crotonobetaine and BB (Seim and Strack, 1980a). Prentiss and colleagues (1961) implicated intestinal microbiota with degrading dietary carnitine to these metabolites.

Under physiological conditions, small concentration of trimethyaminoacetone has been detected in urine that seems to be formed by microbiota degradation of dietary carnitine. Interestingly, oral administration of unphysiological isomer (+) carnitine to rodents induced excretion of trimethylaminoacetone higher than that induced by (-) carnitine administration. This result is suggestive that (+) carnitine is catabolized in mammalian tissues to trimethylaminoacetone by a pathway yet to be unraveled (Seim et al., 1980b). Whereas some degradation of (-) carnitine by mammalian tissues cannot be completely ruled

out this degradation might be of less physiological significance. Thus, it likely that carnitine is mostly generally unchanged in the urine (Stanley, 1995).

Regulation of Carnitine Homeostasis

Regulation of carnitine homeostasis is poorly understood and might be limited. Rat neonates seem to obtain significant amount of carnitine from doe's milk (Robles et al., 1976). Growing rats are able to adequately synthesize sufficient carnitine even when fed carnitine-lacking diets (Borum, 1978) because they possess carnitine biosynthesis enzymes in most of their tissues. In contrast, in human babies, dietary carnitine seems to be essential for normal development (Borum et al., 1981). It has been shown that inadequate dietary lysine decreases carnitine content in epididymis and cardiac and skeletal muscles (Borum and Broquist, 1977). Similarly, it has been shown that dietary carnitine enhances growth of rats fed a low-methionine diet, indicating that dietary carnitine may possibly have a methionine-sparing effect (Khairallah and Wolf, 1965). On the other hand, protein that contains methylated lysine is required for carnitine synthesis (Labadie et al., 1976). Bremer (1961) showed that less of the methionine methyl groups is incorporated into carnitine. Therefore, Dunn and Englard (1981) suggested that availability of carnitine does not interfere with protein methylation. It is evident that the trimethyllysine generated from protein degradation is either converted to carnitine or excreted in urine (Kakimoto and Akazawa, 1970), depending on carnitine tissue concentrations. Thus, production of carnitine from trimethyllysine too may be regulated, but whether this process is influenced by carnitine status is yet to be determined. A study by Holme et al. in 1982 has indicated that the γ-BB and 2-oxoglutarate dioxygenase reaction is not dictated by carnitine status. Because 2-oxoglutarate dioxygenase also converts

trimethyllysine to carnitine, it has been speculated that carnitine status may not regulate trimethyllysine metabolism (Hulse et al., 1978). As previously mentioned, in animals, carnitine is execrated mainly unaltered in urine. Additionally, trimethyllysine excretion is decreased by hypothyroidism but increased in patients suffering thyrotoxic conditions (Maebashi et al., 1977) as protein turnover increases. Kidney failure is known to increase plasma carnitine concentrations (Chen and Lincoln, 1977), suggesting that an active role for the kidneys in regulation of the plasma carnitine concentrations.

Carnitine Deficiencies

Primary carnitine deficiency is triggered by defective plasma membrane carnitine transporters (OCTN2) in muscle and kidney and is predominantly limited to muscular tissues. The lack of the plasma membrane carnitine transporters results in enhanced urinary carnitine excretion coupled with decreased intracellular carnitine concentration (Makhseed et al, 2004). Mutations of *OCTN2* have been shown to cause carnitine deficiency. Myopathic carnitine deficiency is well characterized in muscles and is manifested as severe reduction in muscle carnitine concentrations. The basic etiologic trigger of the primary carnitine deficiency is not identified yet. In contrast, secondary carnitine deficiency is manifested as low tissue or plasma carnitine concentrations. Usually, secondary carnitine deficiency has been found to accompany certain metabolic genetic defects, iatrogenic conditions, or medical disorders. Disturbance of the carnitine metabolic pathway or ß-oxidation of fatty acids has been implicated with causing secondary carnitine deficiency. For instance, repressed fatty acid β-oxidation induces a buildup of acyl-CoA intermediates. Conjugation with carnitine forms acylcarnitine and free CoA. These AC forms are eliminated readily by the kidney and

excreted in urine. In the kidney, AC inhibits carnitine reabsorption by the carnitine transporter associated with renal cells. Subsequently, the increased urinary carnitine causes a systemic secondary exhaustion of carnitine (Scaglia, 2001).

Also, lysinuric protein intolerance is a condition associated with an elevated urine excretion of lysine, the precursor of carnitine. Patients with lysinuric protein intolerance are known to have low ratio of free to total carnitine in their blood, and supplementation with L-carnitine increases their plasma total carnitine (Laura et al., 2008).

Acidemia because of increased acidic molecules in the blood, such as isovalerate, propionate, or methylmalonate, induces accumulation of acyl-CoA intermediates. Subsequently, AC esters accumulate and, thus its execration in urine is increased. As a consequence, total plasma and tissue carnitine concentration decrease, causing secondary carnitine deficiency in children. Carbamoyl phosphate synthase deficiency hinders urea cycle activity and induces carnitine deficiency (Scaglia, 2001). Also, cytochrome c oxidase deficiency impairs the mithochondrial electron transport chain functions and decreases rates of ATP production. Thus, the energy-requiring uptake of carnitine is hindered, causing secondary carnitine deficiency (Scaglia, 2001).

Carnitine homeostasis is affected by other health conditions. For example, long-standing renopathy or liver fibrosis may impair carnitine biosynthesis (Evangeliou and Vlassopoulos, 2003). Low dietary carnitine intake, intestinal conditions associated malabsorption, and health conditions characterized by enhanced catabolism causes secondary carnitine deficiency. Also, because their carnitine biosynthesis is inadequate and their renal carnitine reabsorption is ill developed, preterm neonates are especially at risk for exhibiting carnitine deficiency (Scaglia, 2001). Several medications, such as valporate, emetine, and

zidovudine have been implicated with triggering secondary carnitine deficiency as a side effect. For instance valproate has been suggested to induce secondary carnitine deficiency by several biochemical mechanisms including dissipation of CoA and CoA-related molecules, causing suppression of fatty acid oxidation. In vitro, valporate has impaired uptake of carnitine through plasma membrane of fibroblasts. It is possible that the impaired carnitine uptake via plasma membrane is manifested as lowered serum carnitine and renal reabsorption and thus depletion of carnitine in muscle (Scaglia, 2001). Additionally, dietary vitamin and mineral deficiencies contribute to secondary carnitine development. Stanley and coworkers (1995) have implicated ferrous ion and ascorbic acid deficiencies in causing carnitine deficiencies. Iron and vitamin C are cofactors required for carnitine biosynthesis (Wanders et al., 2002). Collectively, carnitine deficiency is manifested as muscle atrophy and fatigue, cardiac diseases, and neurological disorders. These typical symptoms indicate the biological function of carnitine in energy metabolism.

Effect of Carnitine on Health

Diabetes Type II

Diabetes type II is characterized by dislipedemia and insulin resistance. This is suggestive of impaired β-oxidation (Mingrone et al., 2004) and possible mitochondria malfunction. Recently, Amin and Nagy (2009) reported that the carnitine and herbal extract act as potential anti obesity agents. Carnitine and herbal supplementation decreased hyperglycemia and improved insulin sensitivity when administered to rabbits fed the high fat diet for 14 weeks through enhancing mitochondrial functions.

Similarly, De Gaetano and colleagues (1999) thought that administration of L-Carnitine would ameliorate the dislipedemia and hyperglycemia by enhancing insulin action. Sima et al. (2005) administered groups of subjects known to have either diabetes type I or II with three grams of L-Carnitine given orally for twelve months and observed an alleviated neuropathic symptoms.

Cardiovascular and peripheral arterial disease

Arterial stenosis is a major cause of cardiac ischemia, which is characterized by inadequate blood circulation in the extremities (Hiatt et al. (2004). Supplemental propionyl L-carnitine given to subjects with impaired leg blood circulation at a daily dose of two grams for a whole year significantly improved their endurance to walking (Brevetti et al., 1999). Recently, Sayed and coworkers (2010) have shown that carnitine supplementation in the form of propionyl L-Carnitine prevents the progression of cardiotoxicity through enhancing mitochondrial function Because concentrations of carnitine are low in the failing heart muscle, supplemental amounts might be beneficial to that organ by counteracting the toxic effects of free fatty acids and improving carbohydrate metabolism (Ferrari et al., 2004).

Cancer

Cancer patients are known to be carnitine deficient (Crucuani et al., 2004). Carnitine deficiency might be caused by malnutrition, radiation or chemotherapy (Crucuani et al. (2004) and cause cancer patients to suffer from fatigue. Daily supplemental carnitine at four grms for seven days has been shown to alleviate the fatigue in cancer patients undergoing chemotherapy (Graziano et al., 2002). Additionally, AC enhanced the antitumor effect of cisplatin in human tumor cell line with functional p53. Pisano et al. (2010) have found that AC enhances the stability of p5 and thus sensitivity of the tumor cell lie to the cisplatin

causing significant reduction in the tumor metastasis in vivo. Also, dyslipidemia has been implicated with cellular toxicity, increased mitochondria decay, and generation of reactive oxygen species (**ROS**) that induce oxidative stress. Chronic inflammatory process because of accumulated ROS is involved in the etiology of hepatic carcinoma (Chang et al., 2005). In rat model of hepatocarcinoma, supplemental FC preserved the mitochondrial integrity, reduced generation of ROS, and prevented the development of chronic liver inflammation that initiates the development of hepatic carcinoma (Chang et al., 2005).

Aging

Mitochondrial membrane leakage is actively involved in the process of aging. Supplementation of rat chow with AC and the antioxidant α-lipoic acid reduced the mitochondria leakage by enhancing mitochondrial membrane (Hagen et al., 2002). This was strong evidence that age-induced decrease of carnitine might be trigger of the mitochondrial membrane instability (Ames et al., 2004). Additionally, Bowman and coworkers (1992) hypothesized that aging reduces the uptake of choline and that reduced choline uptake could be associated with Alzheimer's development in elderly people. Further studies by Bowman and colleagues (1992) and Montgomery and colleagues (2003) illustrated that AC supplementation augments the active uptake of choline and the production of acetylcholine and nerve synapses. Subsequently, AC supplementation has improved the Alzheimer's-induced mental incapacity in elderly people.

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS)

HIV-infected individuals are susceptible to impaired lipid metabolism and dyslipedemia and insulin resistance. This metabolic syndrome is further exacerbated by the

anti-HIV drugs that are known to induce carnitine deficiency and subsequent mitochondrial decay (Day et al., 2004). Oral and parenteral administration of carnitine to individuals with AIDS has been shown to slow the decrease of $CD4^+$ T helper cells numbers (Moretti et al., 1998; Mauss and Schmutz, 2001), alleviate the dyslipidemia and slow the advance of the AIDS symptoms.

Liver fibrosis

A role of carnitine as an anti-hepatitis and liver fibrosis nutrient has been suggested (Chang et al., 2005). In agreement with this, supplemental L-carnitine to patients with active liver fibrosis significantly ameliorated dyslipidemia (Mariano et al., 2005). Reduced dyslipidemia is a prerequisite to preventing oxidative stress that sustains chronic hepatitis and fibrous tissue formation.

Male sterility

It has been hypothesized that supplemental carnitine could accelerate fatty acids β-oxidation and thus the capture of ATP by the spermatozoa mitochondria (Ng et al., 2004). In congruent, daily supplementation of sterile individuals with two to three grams of carnitine has enhanced their sperm production (Costa et al., 1994) and motility (Sigman et al., 2006). Additionally, a recent study by Morgante et al. (2010) indicated that treatment with AC and I-arginine improved sperm motility and enhanced sexual performance in infertile men.

Terminal nephropathy

The kidneys are the main human organs that carry carnitine synthesis (Rigault et al., 2006). Thus, nephropathy can significantly alter carnitine concentrations and availability Calvani et al., 2004) through impaired renal carnitine synthesis and reabsorption. Individuals with renal failure are known to acquire carnitine deficiency that initiates an altered lipids

profile and predisposes affected individuals to developing coronary heart diseases. Hurot and colleagues (2002) suggested that effects of carnitine on improving the lipids profile and coronary heart symptoms of individuals with nephropathy warrant more investigation to establish such effects.

Carnitine as a treatment of valproic acid-induced toxicity

Prolonged use of antiepileptic drugs, such as valporic acid might induce toxicity in some susceptible individuals. Valporic acid toxicity is characterized by liver toxicity and elevated blood ammonia concentration that leads to encephalopathy (Lheureux et al., 2005). It has been suggested that L-Carnitine could an effective preventative for the hepatic insufficiency because of valporic acid toxicity (Li et al., 1991). L-Carnitine administration can enhance valporic acid β-oxidation alleviating its heptotoxic effect (Li et al., 1991).

The so-called carnitine shuttle or β-oxidation of valporic acid is well characterized (Figure 5). Like regular β-oxidation of fatty acids, VPA is activated and conjugated to the reduced CoA to form valproyl-CoA. This reaction catalyzed by the ATP-dependent medium-chain acyl-CoA synthetase. Valproyl-CoA is translocated through the outer mitochondrial membrane by the action of the palmitoyl carnitine transferase (**PCT**; Figure 5). Subsequently, valproyl-carnitine is formed in the intermitochondrial. Carnitine translocase facilitate the entrance of valporyl-carnitine to the matrix in substitution to a molecule of L-Carnitine (Lheureux et al., 2005; Figure 5). Eventually, valporyl-carnitine is converted into valporyl-CoA in a reaction catalyzed by PCt-2 that can be β-oxidized reducing the intracellular valporyl-CoA (Lheureux et al., 2005; Lie et al., 1991).

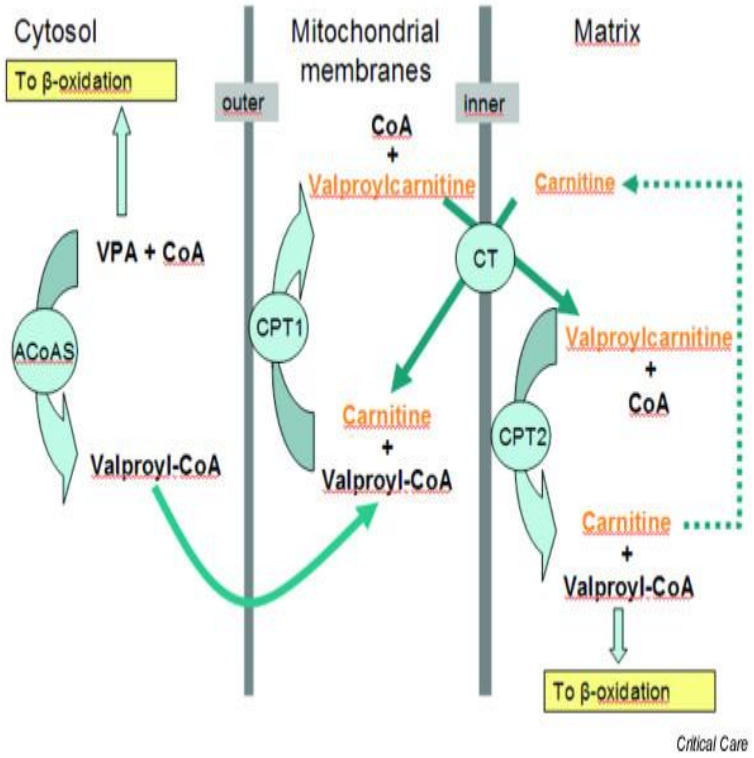

Figure 5. The carnitine shuttle. Acyl-CoA synthetase (ACoAS); coenzyme A (CoA); carnitine palmitoyltransferase (CPT); carnitine translocase (CT). Adopted from Lheureux et al. **(**2005).

Summary

Carnitine (3-hydroxy-4-trimethylaminobutyrate) is dispersed mainly within skeletal and cardiac muscles. Recently, the interest in the metabolism and functions of carnitine has progressively increased. Carnitine is provided to the body by an endogenous biosynthesis from condensation of methionine and lysine and from exogenous dietary sources.

Carnitine is synthesized by the majority of eukaryotic organisms. Carnitine synthesis is initiated by the methylation of lysine. Subsequently, the trimethyllysine formed is converted to butyrobetaine in all tissues. Eventually, the butyrobetaine is hydroxylated to carnitine mainly in the liver. In some animals, however, butyrobetain can be hydroxylated to carnitine in the kidney and brain. The newly synthesized carnitine is usually released from

renal and hepatic tissues to blood stream, where it is taken up actively by all other non-renal and non-hepatic tissues, such as skeletal and cardiac muscles. The regulation of carnitine synthesis is still partly understood.

The primary function of carnitine is to transport activated fatty acids across the inner mitochondrial membrane. Carnitine and carnitine acetyltransferase also are found in the peroxisomes. In peroxisomes, carnitine acetyltransferase is important to transfer the acyl groups, which are produced by the peroxisomal β-oxidation enzymes to the mitochondria for further B-oxidation. The resultant acetyl-CoA is transported to undergo further oxidation in the citric acid cycle.

Congenital anomalies of carnitine metabolism and function have been elucidated. In some patients, the activity of carnitine palmitoyltransferase is decreased, causing muscular fatigue and myoglobinurea. Similarly, reduction of the carnitine palmitoyltransferase activity impairs the ability of the liver to oxidize fatty acids to ketone bodies. The primary and the secondary deficiencies of carnitine in tissues have been shown to impede the ability of tissues to concentrate and retain carnitine. Interestingly, carnitine in the form of acetyl and porpionyl L-Carnitine has been reported to be potential therapeutic agents for the treatment of several diseases that include some neurological, cardiovascular, and metabolic disorders.

Thesis Organization

This thesis is presented as one complete paper, with an abstract, introduction, materials and methods, results, discussion, conclusions, and references, prepared for submission to the *Journal of Animal Science*. The title of the paper is “Variation of Carnitine Concentrations in Angus Beef.” This thesis was prepared from a research carried out to fulfill

the requirements for a Master of Science degree. The first author was responsible for the chemical and data analyses, graphing the data, organizing the results and writing the paper; the co-authors helped with mentoring, data organization, editing, and statistical analyses and gave scientific advice during the study. Data from this experiment suggest that increased carnitine concentration in beef is associated with increase yield grade and marbling score in beef from Angus beef cattle. A thorough literature review precedes the paper. The paper is followed by general conclusions, including recommendations for future studies, and acknowledgments.

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CHAPTER II: VARIATION OF CARNITINE COCENTRATIONS IN ANGUS BEEF

A paper to be submitted to the Journal of Animal Science

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Abstract

The objective was to quantify the naturally occurring free L-Carnitine (**FC**), acylated (**AC**), and total carnitine (**TC**) in skeletal muscle of Angus beef cattle. These data then were correlated to the yield grade (**YG**) and marbling scores (**MS**) of the same samples of longissmus dorsi muscles at 12th rib were collected from 1085 purebred Angus bulls, steers, and heifers from Iowa and California and used to quantify different the forms of carnitine. The spectrophotometric and enzymatic quantification of the different carnitines demonstrated that concentration of FC ranged from 2.12 to 6.32 µmol/g of beef and averaged 3.77 ± 0.80 µmol/g of beef. Also, the AC concentration ranged from 0.05 to 1.61µmol/g and averaged at 0.71 ± 0.17 µmol/g of beef. Similarly, the TC ranged from 2.60 to 7.28 µmol/g of beef and averaged at 4.48 ± 0.88 µmole/g of beef. Overall beef from male Angus beef contained higher FC (P = 0.01) and tended to have higher TC (P = 0.09) than that of beef from female. The AC, however, tended to be higher (P = 0.08) in beef from female Angus beef cattle. Additionally, the yield grade (**YG**) was greater in female Angus beef cattle. The marbling score (**MS**), however, tended to greater (P = 0.06) in beef from male Angus beef cattle compared with that of beef from the female. These data suggest that increased carnitine concentration in beef is associated with increase YG and MS of beef from Angus cattle.

Introduction

L-Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is an essential metabolite, which has an important role in fatty acid catabolism via enhancing transfer of acyl groups across the inner mitochondrial membrane (Borum, 1983). Prior to β-oxidation, the fatty acyl-CoA is esterified with carnitine to form acylcarnitine via reaction catalyzed by acylcarnitine transferase 1 and translocated across the inner mitochondrial membrane by carnitine acylcarnitine translocase action. Next, acylcarnitine transferase Π in mitochondrial matrix frees carnitine. Acyl CoA however, undergoes catabolism via β-oxidation to yield acetyl-CoA as an end product. Eventually, acetyl-CoA undergoes further oxidation via tricarboxylic acid (**TCA**) cycle (Wachter et al., 2002). Intracellular L-Carnitine is present in an acylated and free forms. Although long-chain fatty acid esters of L-Carnitine are transported mainly for β-oxidation, short-chain esters help in regulating the acetyl-CoA/CoA ratio (Shimada et al., 2004).

L-Carnitine concentrations differ considerably in different animal species and tissues (Bremer, 1983). Additionally, L-Carnitine concentrations are influenced by the age, gender birth seasons and feed composition of the diet. It has been shown that the skeletal muscles contain significant L-Carnitine concentration in comparison to other tissues in most animal species (Shimada et al., 2004). The primary and the secondary deficiencies of carnitine in tissues have been shown to impede the ability of tissues to concentrate and retain carnitine (Scaglia, 2001). We analyzed longissmus dorsi muscles from different gender and age groups of Angus beef cattle for different forms of carnitine. We observed significant variation in concentrations of all forms of carnitine longissmus dorsi muscles of Angus beef cattle. In agreement with Borum (1978), we found that male Angus beef cattle, contained higher

carnitine concentrations in their longissmus dorsi muscles than the same muscular from the female cattle. Most important, increased concentrations of carnitine were correlated positively with enhanced beef quality**.**

Materials and Methods

Animals

Beef samples were collected from male and female Angus cattle (n = 2,255) and used to determined free (**FC**), acylated (**AC**), and total carnitine (**TC**) concentrations. The cattle consisted of males and females from the Iowa State University Angus breeding project (Ames, Iowa) and males from collaborating beef farms in California. All cattle were raised with no implants and no antibiotic growth promoters. Beef cattle were slaughtered and tissues were harvested at commercial facilities with an average age of 457 ± 46 days. Longissimus dorsi muscle samples were collected, trimmed of external connective and adipose tissues, freeze-ground, packed, and stored at -20^{o}C until carnitine concentrations were determined.

Reagents

The reagents containing 0.5 M HEPES, 10 mM EDTA of the disodium salt), and 2.7mM of dinitrothiobenzoic acid (**DTNB**; Sigma-Aldrich catalog number D8130) were dissolved in distilled water. By using 1 M sodium hydroxide, the pH was adjusted to about 7.5. The solution was stored at -20^{o} C until used. Acetyl-CoA solution was prepared by dissolving acetyl-CoA (Sigma-Aldrich, catalog number A2056-25MG) in distilled water at final concentration of 11.4 mM and stored at -20^{o} C until used. Then, mixture of 2.4 ml of DTNB and 0.4 ml of acetyl-CoA was prepared every day prior to conducting the analyses.

L-Carnitine standard curve was created by dissolving 12.26 g of L-Carnitine hydrochloride (Sigma-Aldrich, catalog number CO283) in 100 ml of distilled water and stored at -20^{o} C until used. Subsequently, 20 µl 5mg/mL of carnitine acetyltransferase (**CAT**; Sigma-Aldrich, catalog number C4899-10MG) was added to catalyze the reaction.

Carnitine analysis

Quantification of FC in beef tissue is based on the enzymatic conversion of carnitine to acetylcarnitine and coenzyme A (**CoA**) in the presence of excess acetyl-CoA and CAT (Marquis and Fritz, 1964; Xia and Folker, 1991), which is highly specific for L-Carnitine. For carnitine analysis, ground beef was processed according to a protocol modified from Shimada et al. (2004) and Prieto et al. (2006). Briefly, about 1.0 g of ground beef was added with 5.0 ml of 0.3 M perchoric acid and homogenized on ice (POLYTRON; Brinkmann Instruments, Rexdale, Ontario, Canada). Subsequently, the beef homogenate was centrifuged at 8,385g at 4^{o} C for 10 minutes. The homogenate was neutralized with 1.2 M potassium carbonate and filtered through glass wool. The filtrate was used to quantify the FC. TC was quantified after hydrolyzing the AC. The spectrophotometer (SPECTRA Max PLUS, Sunnyvale, CA) was used to determine the optical density at 415 nm before adding CAT to establish base line reading and after 10 minutes of incubation at 37^{0} C after adding CAT. The concentration of carnitine expressed at µmole/g beef was determined from the line equation of the L-Carnitine standard curve.

Statistical analysis

The same statistical model was applied to analyze the variables hot carcass weight (**HCW**), external fat measured at 12th rib (**Fat12**), rib eye are at the 12th rib (**REA12**), the internal fat (perirenal, pelvic, and pericardia; **KPH**), the marbling score (**MS**), the Warner-

Bratzler Shear (**WBS**), and concentration of AC, LC and TC. Factors used in the statistical model were: gender, feeding location, birth-season as fixed effects and the harvest age as the covariate parameter. The Mixed Procedure of SAS (SAS Institute, 2009) was used to fit a general linear model. After normality assumption about residuals was provided using numerical (Kolmogorov-Smirnov test) and graphical (normal probability plot) methods in the SAS, the Univeriate Procedure of SAS was used to provide reliable and valid statistical inference. Subsequently, significant effects were identified in the linear model. Differences between least squared means of factor levels were considered significant at probability value ≤ 0.05 based on the Tukey adjustment type I error rate. The Pearson Correlation Coefficient analysis also was used to correlate the traits under study.

Results

Effects of gender by feeding location interaction on carnitine concentrations

We observed that the FC measured in µmole/g beef was significantly greater (P = 0.01; Figure 1A) in longissimus dorsi muscle from males of Angus beef cattle raised in Iowa in comparison to that of females raised in the same state. Also, the concentrations of TC tended to be greater increase (P = 0.09; Figure 1A) in longissimus dorsi muscle from the same males compared with those of females raised in Iowa. In contrast, the AC concentration tended to be greater (P = 0.08; Figure 1A) in longissimus dorsi of female Angus beef cattle compared with those of the males of the same location.

Noteworthy, Angus beef cattle born in the spring of 2007 contained significantly more FC, AC, and TC (all P = 0.0001; Figure 2C) compared with Angus beef cattle born in the spring of 2005 and 2006. In contrast, Angus beef cattle born in spring of 2005 and 2006 had

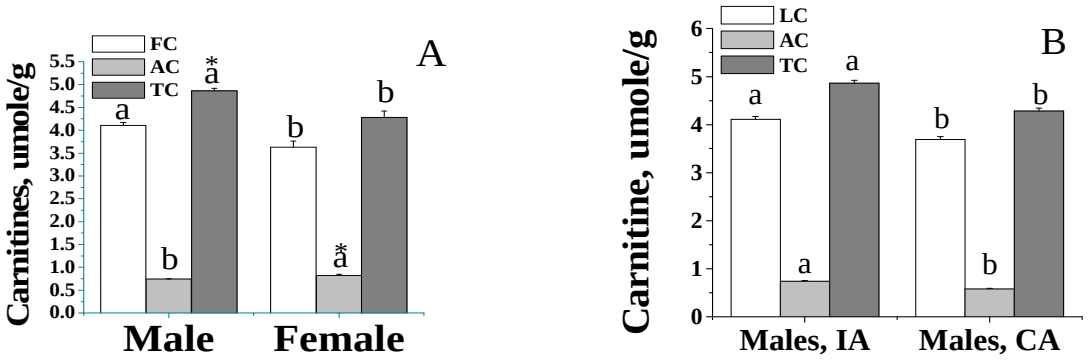

Figure 1. **Panel A. Effect of the gender*feeding location interaction on concentrations of FC, AC, and TC in µmole/g beef in male (n=572) and female (n=154) beef cattle raised in Iowa. Panel B. Effect of feeding location on concentrations of FC, AC, and TC in µmole/g in male Angus beef cattle raised in IA (n=572) and CA (n=360).** Different letters indicate significant difference at $P \leq 0.05$ and different letter with (*) indicates tendency at $0.05 > P < 0.10$.

similar ($P = 0.41$; Figure 2C) FC content. On the other hand, Angus beef cattle born in the fall of 2005 had greater ($P = 0.0001$; Figure 2A and 2B) FC content in their longissimus dorsi muscle compared with those Angus beef cattle born in the fall of 2006 in Iowa.

The content of AC, however, did not differ ($P = 0.98$; Figure 2A and 2B) between Angus beef cattle born in the spring of 2005 and spring of 2006. Similarly, the AC content of the longissimus muscle did not differ ($P = 0.48$; Figure 2A and 2B) between Angus beef cattle born in the fall seasons of 2005 and 2006.

Subsequently, the TC concentration was significantly greater ($P = 0.0001$; Figure 2C) in longissmus dorsi muscle of beef cattle born in spring of 2007 compared with that of Angus beef cattle born in the spring of 2005 and spring 2006. But the concentration of TC did not differ ($P = 0.38$; Figure 2A and 2B) between beef cattle born in springs of 2005 and 2006. In contrast, cattle that were born in fall of 2005 had higher TC content ($P = 0.0001$) compared with that of those cattle born in fall 2006 (Figure 2A and 2B). However, TC concentration

was significantly greater (P = 0.0001; Figure 2A and 2B) in cattle born in fall 2005 compared with that of Angus cattle born in spring 2005. TC concentration tended to be greater (P = 0.07; Figure 2B) in cattle born in the fall of 2006 compared with that of Angus beef cattle born in the spring of 2006. FC concentration was significantly greater (P = 0.0001; Figure 2A) in Angus beef cattle born fall 2005 compared with that of Angus beef cattle born in spring 2005. FC concentration did not differ (P = 0.48; Figure 2B) between beef cattle born

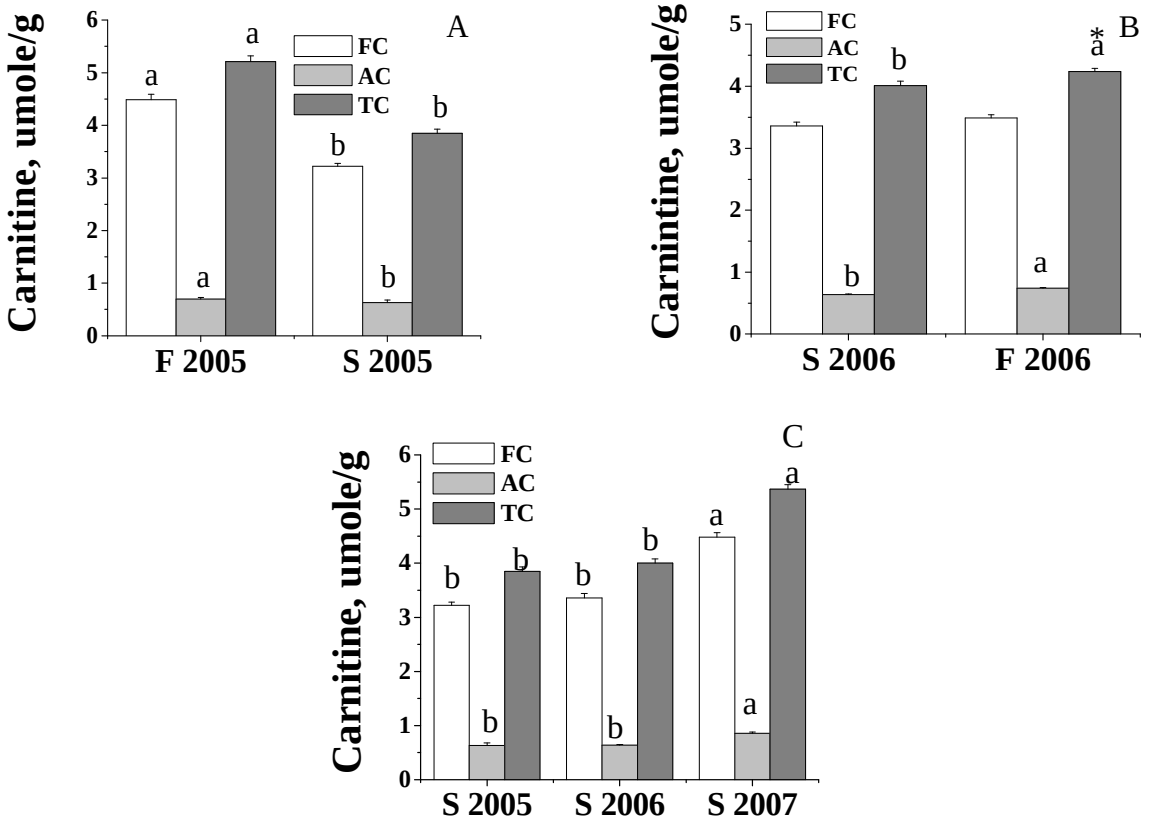

Figure 2. Effect of birth season on concentration of free (FC), acylated (AC), and total carnitine (TC) in µmole/g beef in Angus beef cattle raised in IA and CA. Panel A. Effect of birth in fall (n=60) and spring (n=40) of 2005 seasons on carnitine concentrations. Panel B. Effect of birth in fall (n=43) and spring (n=63) of 2006 seasons on carnitine concentrations. Panel C. Effects of birth in spring 2007 (n=360), 2006 and 2005 on carnitine concentrations. Different letters indicate significant difference at $P \leq 0.05$ and different letter with (*) indicates tendency at $0.05 > P < 0.10$.

in fall of 2006 compared with that born spring 2006. In addition, AC content was significantly greater ($P = 0.02$ and $P = 0.0001$, respectively; Figure 2A and 2B) in beef from Angus beef cattle born in fall of 2005 and 2006 compared with those beef from Angus beef cattle born in springs of 2005 and 2006.

Noteworthy is that male Angus beef cattle raised in IA demonstrated greater FC, AC, and TC concentrations (all $P = 0.0001$; Figure 1B) compared with the males of the same breed raised in CA. Similarly, REA12 and HCW were greater ($P = 0.002$ and $P = 0.0001$, respectively; Table 1) in Angus male beef cattle raised in IA compared with that of beef from Angus males beef cattle raised in CA. In addition, Fat12 tended to be lesser ($P = 0.09$; Table 1) in beef from Angus males beef cattle raised in IA compared to those beef from Angus males cattle raised in CA. Also, KPH was significantly lower ($P = 0.006$, Table 1) in Angus male beef cattle raised in IA compared with that of beef from Angus males beef cattle raised in CA. In contrast, WBS was significantly greater ($P = 0.001$, Table 1) in Angus male beef cattle raised in IA than those of beef from Angus males beef cattle raised in CA.

Table 1. Effect of feeding location on different carcass qualities in Angus beef cattle.

	Feed location		Probability
Trait	IA(n=572)	CA (n =360)	*P*-value
HCW, lb	754 ± 5.6	724 ± 6.02	0.0001
Fat12, inch	0.45 ± 0.01	0.49 ± 0.01	0.09
KPH, rel. score	2.18 ± 0.02	2.29 ± 0.02	0.006
REA12**,** inch^2	12.93 ± 0.09	12.45 ± 0.1	0.002
WBS, Kg	3.52 ± 0.05	2.97 ± 0.05	0.001
FC, µmole/g	4.11 ± 0.06	3.67 ± 0.06	0.0001

Note. External fat at rib 12 (**Fat12, inch**); rib eye are at rib 12 (**REA12, inch^2**); hot carcass weight (**HCW, lb**); yield grade (**YG, rel. score**); marbling score (**MS, rel. score**); Warner-Bratzler Shear force (**WBS, kg).**

Effects of gender by feeding location interaction on beef quality traits

In female Angus beef cattle, the concentration of AC tended to be greater (P = 0.08; Figure 3A) compared with that of male Angus beef. Simultaneously, Fat 12 thickness was lower (P = 0.0001; Figure 3A) in female Angus beef. The KPH was not altered (P = 0.13; Figure 3A) with the increase of AC. In addition, REA12 in male Angus beef was significantly greater (P = 0.04) compared with that of female Angus beef. Of importance, the HCW of female Angus beef was significantly lower (P = 0.0001) than that of the males of the same breed. The MS tended to increase (P = 0.06; Figure 3B) in males Angus beef compared to that of the females. Similarly, the concentration of the AC tended to increase (P = 0.08; Figures 3A and 3B) in female Angus beef compared to that of the males of the same breed. The WBS force, however, was not different (P = 0.60) between males and females Angus beef cattle (Figure 3B). These results indicate that YG was positively correlated (r = 0.31, P = .00001, r = 0.12, P = 0.0001, r = 0.3, P = 0.002 respectively; Table 2) with FC, AC, and TC. On the other hand, MS was positively correlated (r = 0.2, P = 0.0001, r = 0.18,

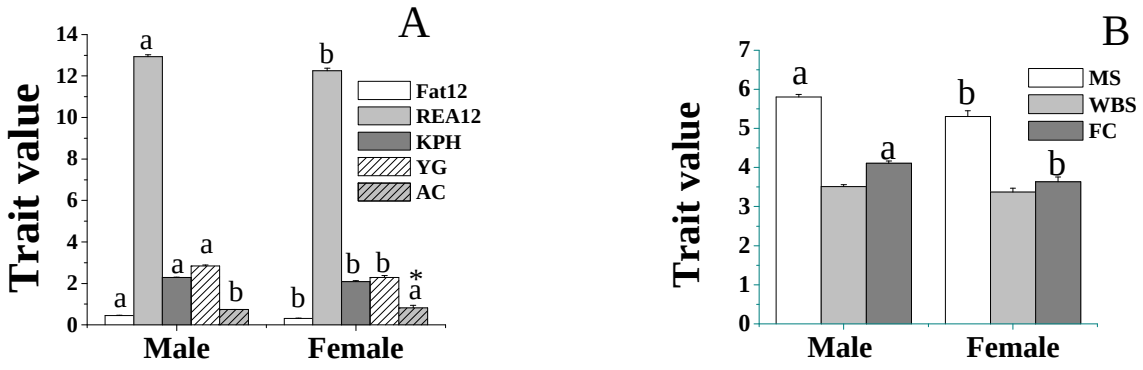

Figure 3. Panel A. Relationship of AC, µmole/g to beef quality traits external and internal fat (Fat 12, inch and KPH, rel. score, respectively), and rib eye area (REA12, inch^2) in male (n=572) and female (n=154) Angus beef cattle. Panel B. Relationship of FC (µmole/g) to marbling (MS, rel. score) and tenderness (WBS, kg) of beef from male (n=572) and female (n=154) Angus beef cattle. Different letters indicate significant difference at $P \leq 0.05$ and different letter with (*) indicates tendency at $0.05 > P < 0.10$.

Table 2. Correlation between carnitine concentration and carcass qualities in Angus beef cattle.

	Traits							
	HCW	Fat12	REA12	KPH	WBS	FC	AC	TC
YG	0.4	0.89	- 0.49	0.16	0.15	0.26	0.08	0.25
P-value	≤ .0001	≤ .0001	≤ .0001	0.0002	≤0.0001	≤ .0001	0.03	≤0001
MS	0.11	0.28	- 0.14	0.04	- 0.11	0.11	0.04	0.12
P-value	≤ .001	≤ .0001	≤ .0001	0.24	0.002	≤ .001	0.06	≤ .001

Note. Free carnitine (**FC, µmole/g**); acylated carnitine (**AC, µmole/g**); total carnitine (**TC, µmole/g**); external fat at rib 12 (**Fat12, inch**); rib eye are at rib 12 (**REA12, inch^2**); hot carcass weight (**HCW, lb**); yield grade (**YG, rel. score**); marbling score (**MS, rel. score**); Warner-Bratzler Shear force (**WBS, kg**); internal fat (perirenal, pericardial, and pelvic fat; **KPH, rel. score**).

P = 0.0001; Table 2) with FC and TC. MS, however, did not correlate (r = 0.04, *P* = 0.25; Table 2) with AC.

Discussion

We noticed that the FC was significantly greater (*P* = 0.01; Figure 1A) whereas TC tended to be greater (*P* = 0.09; Figure 1A) in longissimus dorsi muscle from males of Angus beef cattle raised in IA in comparison to those in muscles from females raised in the same state. Previously, Borum (1978) reported that gender of animal affects carnitine concentration in tissues. For instance TC was greater in plasma and cardiac and skeletal muscles of male rats compared with concentration in female rats. In females, carnitine is secreted into milk and its concentration in cow's milk is 0.1-0.5 mM (Erfle et al., 1974). In addition, hepatic and urinary TC was higher in female rats that in male rats (Borum, 1978). Difference in TC concentration between males and females were apparent with the increase of epididymal carnitine concentration in males. Also, it has been suggested that the age and gender of the animal must be considered when determining carnitine concentration in tissues

(Erfle et al., 1974; Borum, 1978). Generally, the concentration of FC in longissmus dorsi muscles of beef cattle is greater than that of dairy cattle. For instance, in Angus × Hereford and Japanese Black beef cattle at 32 months of age, FC carnitine concentrations averaged 3.47 ± 0.04 and 3.57 ± 0.20 µmole/g longissmus dorsi muscle respectively, (Shimada et al., 2004). But in Holstein steers at ages of 24 and 12 months, the FC concentrations averaged 2.04 ± 0.15 and 1.86 ± 0.49 µmole/g longissmus dorsi muscle (Shimada et al., 2004). This variation of FC in different cattle breeds and ages suggests that FC probably is greater in beef cattle over that of dairy cattle. In addition, it seems that FC increase with age. This conclusion was supported by the finding that FC in 36 months old Holstein dairy cows did not exceed 2.73 µmole/g beef (Shimada et al., 2004), which is slightly lower than that of beef cattle at a similar age; by the age of 60 months, FC in beef cattle was only 2.97 µmole/g beef.

Interestingly, the concentrations of AC tended to be greater ($P = 0.08$; Figure 1A) in longissimus dorsi muscle of females compared with those of males raised in IA. Concurrently, external fat (Fat12) and internal fat (KPH) decreased ($P = 0.0001$, $P = 0.005$ respectively; Figure 3A). In addition YG decreased ($P = 0.0001$; Figure 3A) compared with those Angus beef males. Thus, elevated AC might be associated with increase of leanness and improved YG.

On the other hand, the MS tended to be greater ($P = 0.06$) in male Angus beef cattle in comparison with that of female Angus beef cattle. Because the greater concentration ($P = 0.01$) of FC and the tendency ($P = 0.09$) of TC to be greater were accompanied with a greater MS in beef from Angus males ($r = 0.11$, $P \leq 0.0001$ and $r = 0.12$, $P \leq 0.0001$, respectively; Table 2) in comparison to that of the females. Thus, it is likely that FC is associated with enhanced MS, REA12 and HCW (Table 2). It has been suggested that increased marbling or

intramuscular fat could “dilute” the protein in a given portion of meat, thereby, lowering the bulk density and resulting in an increased tenderness (Savell et al., 1987). Furthermore, the fat deposited within the muscle cells or connective tissues may “thin” the connective tissues to decrease the WBS force (Savell et al., 1987). In agreement with this, we observed that greater FC and TC in beef from males Angus cattle is associated with greater MS (r = 0.11, *P* = 0.0001 and r = 0.12, *P* = 0.0001 respectively; Table 2). In support of this observation, supplementation of FC to Angus-cross steers fed corn-based diet by Greenwood et al. (2001) increases the MS and YG. The increased AC concentration might be associated with accelerated ß-oxidation of fatty acids (Hoe et al., 2000) in female Angus beef cattle. Additionally, female piglets have greater proportion of red fiber muscles and smaller proportion of white fiber muscles than do males (Losel et al., 2009). Red muscles tend to have more lipids in mitochondria and contain higher amount of carnitine to facilitate adequate ß-oxidation (Losel et al., 2009). We examined the effect of birth season on the concentrations of different chemical forms of carnitine in Angus beef cattle. It was evident that concentrations of the different types of carnitine are affected by birth season (Figure 2B). Concentration of all carnitine forms were greater in beef from Angus cattle born in spring of 2007 compared with that of beef cattle born in the spring of 2005 and 2006 (both *P* = 0.0001; Figure 2B). In contrast, there was no difference (*P* = 0.41, *P* = 0.38 and *P* = 0.98 respectively; Figure 2B) between FC, TC and AC of spring 2005 and 2006. In contrast, AC was significantly greater (*P* = 0.0001) and TC tended to be greater (*P* = 0.07) in beef from cattle born in fall of 2006 compared with those born in spring of 2006. On the other hand, FC did not differ (*P* = 0.48) between cattle born in fall and spring of 2006. Interestingly, the concentrations of different forms of carnitine in beef from cattle born in fall of 2005 was

significantly greater ($P \leq 0.02$; Figure 2B) than that of beef from cattle born in spring of 2005. In agreement, mitochondria from red muscles of rainbow trout acclimatized to cold temperature of 1^0 C had increased carnitine palmitoyltransferase 1 activity compared with mitochondria from red muscles of rainbow rout acclimatized to warmer temperature of 16° C (Guderley, 2004). Presumably, this adaptation greater rates of fatty acids oxidation, additionally, we observed that beef from male Angus beef raised in state of IA contained higher concentrations of different forms of carnitine (all $P = 0.0001$; Figure 1B) in comparison with those raised in CA, indicating that carnitine concentrations is likely affected by the average ambient temperature.

We examined the effect of feeding location on different carcass qualities that dictate the yield grade, marbling score, and leanness (Table 1). Angus beef cattle raised and fed in IA had higher concentration of FC, AC, and TC (Figure 1B). The greater of concentration of different carnitine forms tended ($P = 0.09$) to decrease Fat12 and significantly decreased ($P = 0.006$) KPH in beef from female Angus beef. In contrast, the increase of the concentrations of different forms of carnitine was associated with increased ($P = 0.002$) REA12 and HCW ($P = 0.0001$; Table 1), indicating improvement of leanness of beef from Angus cattle raised in IA. In agreement with our data, dietary supplementation of carnitine to growing finishing pigs increased protein accretion and percentage of leanness and decreased fat deposition (Owen et al., 1996; Hoe et al., 2000). Increased protein accretion was expressed as an increase in muscles mass. Similarly, carnitine supplementation at 400 mg daily from day 7 to day 27 of age to piglet of low birth weight decreased the perirenal fat (part of KPH) and increased the total number of myofibrils in semitendinosus muscle by about 13%. Additionally, the carnitine supplementation increased the mRNA expression of the gene

encoding the embryonic isoform of the myosin heavy chain in the semitendinosus muscle (Losel et al., 2009).

Conclusion

The data presented here suggest that FC and TC are greater in beef from of male Angus beef cattle than in beef from female Angus beef cattle and are closely associated with intramuscular fat accretion and, thus, enhance marbling score and tenderness. On the other hand, AC is greater in beef from female Angus beef cattle and is associated with less Fat12 and greater leanness of beef and, thus, improved yield grade. Also, the data suggest that FC, AC, and TC concentrations are increased during low ambient environmental temperature concurrently with the accelerated β-oxidation of fatty acid intended to generate more heat to maintain normal body temperature.

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CHAPTER III. GENERAL CONCLUSIONS

Carnitine (3-hydroxy-4-trimethylaminobutyrate) is mainly distributed within cardiac and skeletal muscles. Lately, the interest in the metabolism and roles of carnitine has been significantly increasing. Carnitine need of the body is sufficed either endogenously by synthesis from primary precursors or exogenously from dietary sources

Endogenously, carnitine synthesis is accomplished by the methylation of lysine and it primarily occurs in the liver. Also, synthesis of carnitine has been indicated in kidney and brain in some animal species. Synthesized carnitine from liver, kidney, and brain is released in the circulation where it is taken up by other tissues. The regulation of carnitine synthesis is still poorly understood.

Congenital carnitine metabolic and functional disorders have been elucidated. These disorders are mainly associated with decreased activity of carnitine palmitoyltransferases. The primary and the secondary carnitine deficiencies in tissues are known to hinder the ability of tissues to obtain enough carnitine.

The short-chain acylated form of carnitine possesses remedial potential and has been successfully used to effectively treat numerous diseases. In this study, we were interested in determining concentrations of different forms of carnitine in Angus beef. To attain this objective we used enzymatic and spectrophotometric quantification to determine the concentration of different carnitine forms. The concentration of FC averaged 3.77 ± 0.80 µmol/g of beef, AC concentration averaged 0.71 ± 0.17 µmol/g of beef, and the TC concentration averaged 4.48 ± 0.88 µmole/g of beef.

Our data suggest that FC and TC are greater in of male in beef from male Angus beef cattle than in beef from female Angus beef cattle, and are closely and positively associated

with intramuscular fat accretion. Thus, carnitine concentrations may dictate the marbling score (**MS**) and degree of beef tenderness. On the other hand, AC has been found to be greater in beef from female Angus beef cattle, and is intimately associated with less external fat deposit (Fat12) and greater leanness of beef and. Thus, elevated AC concentration is implicated with improved yield grade (**YG**). The MS, however, tended to increase ($P = 0.06$) in male Angus beef compared with that of the females. Also, the data suggest that FC, AC, and TC concentrations are effected by low ambient environmental temperature that accelerates the β-oxidation of fatty acids intended to generate heat energy to preserve normal body temperature.

Recommendations for Future Studies

In this study, we investigated the variation of carnitine concentrations in longissimus dorsi muscle of beef cattle. We observed strong association between gender, feeding locations, birth season, and carnitine concentrations. It is evident that carnitine concentrations correlate with fat deposit, YG, and MS. These novel results ignite the curiosity about what genes are associated with this variation and whether these genes can be activated to increase these traits that would improve the healthfulness of beef. Therefore, it is highly recommended that single polymorphism nucleotides (SNPs) that influence different forms of carnitine concentrations and subsequently dictate the YG, MS, and tenderness of beef warrant further determination. Additionally, the nature of the fatty acids that predominate in the external and intramuscular fat (marbling) and are associated with increased carnitine concentrations should be determined and their atherogenic index quantified to gain better insight on how carnitine improves the healthfulness of beef.

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