Carnitine Metabolism and Human Nutrition

offers a contemporary and in-depth look at the biological effects of carnitine metabolism and its application to clinical and sports nutrition based on decades of robust scientific enquiry. It gathers and distills key results of the last 20 years of carnitine research to provide an invaluable reference tool for students, researchers, and clinicians.

This book addresses the importance of carnitine in skeletal muscle fuel metabolism, the complexities and importance of muscle carnitine transport, and the metabolic insight that has been gained from experiments manipulating muscle carnitine stores.

The authors cover the potential application of carnitine supplementation in specific clinical populations and the role of carnitine as an ergogenic aid for athletes. They also provide a comprehensive mechanistic overview of skeletal muscle insulin resistance, including the role of carnitine shuttle systems in the metabolic abnormalities associated with obesity and the metabolic syndrome.

Carnitine Metabolism and Human Nutrition provides you with a comprehensive and up-to-date look at the properties and underlying metabolic biochemistry of carnitine. The book includes contributions from leading international scientists, each a pioneer in their chosen study of carnitine metabolism or its application to human nutrition.

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Preface

Specific molecules or compounds often attract significant media or scientific attention in the world of nutrition. Undoubtedly, this enthusiasm stems from the desire to discover a “magic bullet” and, oftentimes, the ability to commercialize a specific nutritional product. Example goals include weight loss, delaying physical or mental fatigue (e.g., sports performance), countering undesirable effects of illness, combatting the aging process, battling the common cold, improving general health, etc. Invariably, such approaches are based on solid theoretical rationale. However, while a sound scientific rationale is a vital prerequisite for demonstrating efficacy, alone it is insufficient.

Frequently, a specific compound is identified as having a key role within a metabolic pathway, be it a rate-limiting enzyme, enzymatic cofactor, fuel store/substrate, or signaling molecule. It is therefore assumed that ingesting large amounts of “compound X” will result in more efficient flux through key reactions, accumulation of metabolic fuel stores, or the “switching on” of complex metabolic processes. Unfortunately, these assumptions are made prior to the necessary scientific steps being taken to test the theoretical basis of the effect of compound X on real-world outcomes. That is, what happens when it is ingested—what are the pharmacokinetics/bioavailability? How does the kidney deal with excess amounts of the nutrient? In which biological tissues would these effects be expected to occur? Does it reach or enter the target tissues? What are the effects on the metabolic pathways in question? Do any metabolic effects result in meaningful/beneficial effects on physiology, health, or performance? Do any meaningful effects occur acutely, chronically, or both? Does a dose–response relationship exist? Are there any negative side effects? The list could go on indefinitely, but the point is that for any new nutritional therapy to be developed, all these questions and more should be addressed thoroughly by the rigors of the scientific method. Unfortunately, with the surplus of new products and approaches within human nutrition, these steps are often bypassed due to the eagerness to rush into application. This can leave us swimming in a sea of products and approaches with vacuous and unsubstantiated claims. The resultant
confusion for scientists, practitioners, and particularly the public can make practical nutrition a complex exercise.

Although carnitine as a food supplement (and the basis of a multi-million-dollar supplement industry) has been around for decades, this has largely been based upon sound theoretical background followed by assumptions, misconceptions, and marketing. However, over the last two decades, carnitine has been subjected to the rigors of robust scientific enquiry. Though the story is far from complete, and many key questions remain, considerable data have accumulated to demonstrate carnitine’s potential as a legitimate functional food in numerous populations. Early in our academic careers, we had the privilege to have been involved in some of this research, and contribute to the knowledge base that is available today. Editing this book, therefore, has been a labor of love with respect to amalgamating the latest thinking of the place that carnitine occupies in modern nutrition. We were fortunate enough to have contributions from leading scientists across the world, all of whom are pioneers in their chosen study of carnitine metabolism or application of carnitine to human nutrition. Accordingly, we have compiled this book to offer a contemporary and in-depth look at a highly specialized and specific area of human nutrition, and the underlying metabolic biochemistry. We hope that students, scientists, clinicians, nutritionists, and other health professionals alike will share in our enthusiasm for this topic and find this book a useful resource.
Acknowledgments

The editors express their gratitude to all colleagues, mentors, and specifically the contributing authors. Such a specialized book would not be possible without contributions from those esteemed scientists who have generated the data we have available today. Each author was invited due to his or her eminence in the field, and our admiration for each was even greater following our communications over their respective chapters. Additionally, the editors thank Dr. Francis Stephens, whose assistance and consultation in the editing process were invaluable. Benjamin also thanks Mandy, whose love, support, and understanding always shine through and keep the important parts of life in beautiful perspective.
Introduction

Benjamin T. Wall and Craig Porter

Over a century ago, the compound carnitine was discovered in meat extracts [1] and was named *carnis*, meaning “flesh” in Latin. Twenty years later, carnitine was chemically classified as 3-hydroxy-4-N,N,N-trimethylaminobutyric acid, a small water-soluble quaternary amine synthesized from the amino acids methionine and lysine [2]. The biological importance of carnitine began to be recognized in the 1950s when it was identified as an essential growth factor in *Tenebrio molitor* beetle larvae and labeled vitamin B₇ [3]. Pioneering work over the subsequent two decades from Friedman, Fritz, Childress, and others began to elucidate the role of carnitine in energy metabolism, both as an essential cofactor for mitochondrial fatty acid translocation [4–15] and as a cellular buffer to maintain the ratio of mitochondrial acetyl-coenzyme A to coenzyme A [16–20]. Throughout the last two decades of the twentieth century, much of this research began to be translated into human studies investigating carnitine metabolism [21–25]. Such work cemented the understanding of how carnitine behaves in human metabolism, and thus laid the groundwork for investigations into the role of carnitine in human nutrition.

While carnitine is ubiquitously present throughout the tissues of the body, the vast majority (>95%) is located within skeletal muscle. Moreover, by virtue of its mass and role in locomotion, skeletal muscle represents (quantitatively) the primary organ of energy production and consumption. Thus, while carnitine fulfills its vital metabolic roles across all biological tissues of the body, skeletal muscle has been an understandable focus when considering the potential role for carnitine in human nutrition. Numerous metabolic disease states are characterized by a disturbance in skeletal muscle fat or carbohydrate metabolism, which can be either a cause or a consequence of the particular condition. In addition, exercise, from either a health promotion or sports performance viewpoint, leads to a greater requirement for skeletal muscle energy production from endogenous fat and carbohydrate stores. It is under such conditions (i.e.,
metabolic disease or exercise) where the relevance of carnitine administration in optimizing energy production becomes apparent.

The present book aims to provide a comprehensive text describing the modern scientific understanding of carnitine’s function in human metabolism and its application to both clinical and sports nutrition. The first section of the book takes a basic science approach, discussing the tightly regulated homeostasis of carnitine in humans, and its principal biochemistry in key biological tissues. The second section emphasizes the importance of carnitine in skeletal muscle fuel metabolism, the complexities and importance of muscle carnitine transport, and the metabolic insight that has been gained from experiments manipulating muscle carnitine stores. The final section addresses various conditions, in health and disease, where normal carnitine metabolism becomes disturbed and the subsequent rationale and (potential) efficacy of carnitine administration as a beneficial nutritional strategy. It is our hope that this text will provide a comprehensive account of the current understanding of the role carnitine plays in skeletal muscle metabolic regulation, while identifying knowledge gaps in the literature and thus stimulate further robust research in this field.

References
About the editors

Benjamin T. Wall was born in Sheffield in the United Kingdom in 1984. Due to a keen and lifelong interest in health, nutrition, and sports, he chose to follow an undergraduate education in exercise sciences/physiology at the University of Birmingham in the UK. After a brief stint working at the University of Birmingham, Benjamin moved to follow his Ph.D. research at the School of Biomedical Sciences at the University of Nottingham in the UK, in the muscle metabolism research laboratory of Professor Greenhaff. Here, he undertook a series of human studies aimed at understanding the metabolic roles of carnitine and coenzyme A within resting and exercising skeletal muscle. These studies led to the first demonstration of carnitine loading in human muscle via dietary means, and established the distinct roles of each of these essential metabolic cofactors within carbohydrate and lipid metabolism in vivo. Benjamin is now based at Maastricht University in The Netherlands, where he occupies a postdoctoral research fellow position. His present research continues to examine skeletal muscle metabolism, now with a specific focus of the role that nutrition and (in)activity/illness play in human aging. In this field, Benjamin has applied stable isotope methodology and molecular analytical techniques to publish numerous papers describing the underlying biochemical mechanisms governing the regulation of muscle mass and metabolism in humans.

Craig Porter was born in Edinburgh in 1985. Growing up in the suburbs of Scotland’s capital city, he took a keen interest in sport and, in particular, rugby. It was this involvement in rugby during the sport’s transition to its professional era that spawned his interest in physiology and nutrition. In 2003, Craig began his undergraduate education at the University of Glasgow, Scotland, graduating in the fall of 2007 with a first-class honors degree in physiology, sports science, and nutrition. Keen to pursue his Ph.D., Craig relocated to Nottingham, England, to begin a research position in Professor Greenhaff’s laboratory, where he researched the impact of skeletal muscle carnitine depletion on fuel metabolism. Craig successfully defended his Ph.D. in 2011, after which he began a research fellowship in
the Department of Surgery, University of Texas Medical Branch, Galveston. He is currently a member of the scientific staff and associate director of the Metabolism Unit at Shriners Hospital for Children–Galveston, where his research focuses on the role of altered mitochondrial function on the pathophysiological stress response to trauma.
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section one

Carnitine homeostasis and tissue metabolism
chapter one

Carnitine homeostasis in humans

Peggy R. Borum

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Introduction

The many roles of carnitine in metabolism make carnitine homeostasis in humans critically important for maintaining health and treating disease. We are now beginning to recognize that there is also a critically important role of carnitine in human homeostasis. Homeostasis is a self-regulating process that maintains stability while adjusting to changing conditions. Ideally the carnitine metabolite pool and the metabolic processes of the body exist in their own dynamic equilibrium. They reach a balance in which internal change continuously compensates for external change. Thus, the concentrations and locations of the different carnitine metabolites in the body must change to meet the changing sources of carnitine, the interconversion of different acylcarnitines, and the removal of carnitine from the body. Carnitine likely functions in maintaining homeostasis
in many metabolic pathways and physiological conditions, with carnitine’s role in energy metabolism homeostasis being the best studied. The following chapters provide details concerning carnitine transport and metabolism in specific tissues under various physiological conditions, including health, exercise, and disease. The purpose of this chapter is to provide a brief overview of some of the basics of carnitine synthesis, transport, and function, but more importantly, to step back and look at the gestalt of carnitine and then to stand on tiptoes to try to see over the horizon of established carnitine information.

Sources of carnitine

Endogenous biosynthesis of carnitine

Carnitine is synthesized by humans from the essential amino acids lysine and methionine requiring the vitamins riboflavin, vitamin B6, and vitamin C [1]. Thus, malnutrition impacts endogenous carnitine biosynthesis. Many organs have the enzymes needed for the synthesis of gamma-butyrobetaine, which is the immediate precursor of carnitine. Human liver, kidney, testes, and brain have significant gamma-butyrobetaine hydroxylase activity to make carnitine. Since not all mammals have the same tissue distribution of carnitine biosynthetic enzymes, care must be taken in extrapolation of data from one species to another. For example, significant gamma-butyrobetaine hydroxylase activity is not found in rat kidney.

Exogenous sources of carnitine

Dietary carnitine has been considered the major exogenous source of carnitine. Animal foods have higher concentrations of carnitine than plant foods. Since type 1 muscle fibers have higher concentrations of carnitine than type 2 fibers, the redder the meat, the higher the carnitine concentration. Thus, lamb, beef, pork, chicken, and fish have carnitine concentrations in descending order. Even within poultry products, the dark meat has approximately five times the carnitine concentration found in white meat. Plant foods that are fermented (such as tempeh) may acquire carnitine that is synthesized by the fermenting organism. The dietary intake of humans is thus dependent on the type of diet eaten, but does not exceed the 2–5 mg/kg body weight/day range. The bioavailability of dietary carnitine is also influenced by the food source and the preparation of the food. Breast-fed babies likely receive the most bioavailable source of dietary carnitine.

Purified carnitine has become available in the last few decades as nutritional supplements and as pharmaceutical agents. Carnitine provided in concentrations similar to what humans have historically obtained from
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dietary sources is termed a carnitine nutritional supplement. Carnitine provided in concentrations higher than what humans have historically obtained from dietary sources or provided via routes other than the gastrointestinal tract (such as intravenous carnitine) is termed a carnitine pharmaceutical.

Although generally ignored until very recently, carnitine synthesis by our gastrointestinal microbiota is another source of exogenous carnitine. There might be some argument that our gastrointestinal tract microbiota is actually a long neglected organ, and thus is an endogenous source of carnitine. However, for this discussion it is considered an exogenous source of carnitine.

Whether exogenous carnitine is required for the human is a topic of continuing investigation. The potential use of carnitine in a variety of disease states will be discussed in several chapters of this book. Throughout the history of mankind, babies in utero have accumulated needed carnitine from mother, especially during the last trimester of gestation. After birth, the breast-fed baby continues to receive dietary carnitine. During the last hundred years, babies born at earlier and earlier gestational ages have been surviving in our neonatal intensive care units (NICUs). Babies born at 24 weeks gestation do not have the benefit of the last trimester accumulation of carnitine, have a gastrointestinal tract that cannot absorb many nutrients even if provided via tube feeding, and are fed parenterally with solutions that often contain no carnitine. Life in the NICU is much harder than life in utero in many other ways. Yet these babies still have most of their brain to make and many other organ systems that need to mature. Carnitine deficiency is a problem, but excess exogenous carnitine can also be a problem. In addition, the amount that is perfect for one preterm baby may be very wrong for the next baby that looks the same according to our current criteria. What amount of what acylcarnitine(s) to supplement needs to be determined for each baby, and we do not know exactly how to do this [2].

Interconversion of different acylcarnitines

Conversion of acylCoAs to acylcarnitines and back again

Proteins (carnitine transporters) are required to transport carnitine across membranes to the site where it is needed to esterify a carboxylic acid catalyzed by another protein (carnitine acyltransferase). Often the acylcarnitine is transported across membranes to a different site where another carnitine acyltransferase hydrolyzes the ester. An important point is that the substrate for the carnitine acyltransferases is a carboxylic acid activated to the coenzyme A (CoA) form, and thus is a high-energy compound often created at the expense of an adenosine triphosphate (ATP). The beauty of the mechanism of the carnitine acyltransferases is that the acylCoA can
be converted to acylcarnitine and then hydrolyzed to form carnitine and acylCoA without the need for an ATP. For example, acetylCoA can be synthesized at one location at the expense of an ATP, converted to acetylcarnitine, transported to another location, and converted to acetylCoA with no requirement for an ATP at the new location. The new location has not only acquired the needed carboxylic acid, but also acquired it in an active form with no high-energy expense. During critical illness, this characteristic can become important. The carnitine acyltransferases are found in all cell types of the human body with different enzymes preferring substrates of different chain lengths. Thus, the proteins needed for formation, transport, and hydrolysis of carnitine and acylcarnitines appear to have been preserved during evolution.

**Fatty acid metabolism**

The first well-defined function of carnitine was the transport of long-chain fatty acids into the matrix of the mitochondrion for beta-oxidation. Compartmentalization within the cell contributes to homeostasis of the human cell. Separation of fatty acid synthesis in the cytoplasm from fatty acid oxidation in the mitochondria facilitates regulation and coordination of these processes. Carnitine plays an important role in getting the fatty acid into the mitochondrial matrix, and the inhibition of the carnitine palmitoylacyltransferase by malonylCoA (an early metabolite in fatty acid synthesis in the cytosol) also functions in the coordination of the two pathways.

Although carnitine’s role in beta-oxidation in mitochondria is usually emphasized, carnitine also plays a critical role in peroxisomal beta-oxidation by removing the shortened fatty acid from the peroxisomes and transporting them to the mitochondria for further oxidation. Omega-oxidation of fatty acids in the endoplasmic reticulum produces dicarboxylic acids, which can also be attached to CoA and esterified to carnitine.

Carnitine has roles in lipid metabolism in addition to fatty acid oxidation. It has been known for some time that carnitine functions in membrane phospholipid turnover in human erythrocytes [3], which has implications for both increasing the life span of circulating erythrocytes during certain disease states and storage in blood banks [4].

AcetylCoA is highlighted in biochemistry textbooks as a central metabolite. AcetylCoA is a substrate for or a product of many metabolic pathways. In addition, acetylCoA functions in the allostERIC regulation of proteins as well as the stimulation or inhibition of signaling pathways coordinating several metabolic pathways [5]. Phosphorylation and dephosphorylation of proteins are frequent regulatory mechanisms that can be influenced by acetylCoA’s effect on some protein kinases. It is now recognized that acetylation and deacetylation of proteins are other
important regulatory mechanisms, and acetylCoA has an important role. AcetylCoA is sequestered in the cell and in subcellular organelles because it cannot be transported across membranes. If it is at too high a concentration at one location, carnitine can facilitate transportation of the activated acetate from that location. The conversion of acetylCoA to acetylcarnitine will immediately reduce the acetylCoA concentration and will also release free coenzyme A, which may be present in limiting amounts and required to meet immediate needs. If acetylCoA is at too low a concentration at one location, carnitine can facilitate transportation of the activated acetate into that location.

**Carboxylic acids in metabolism**

Numerous metabolites in amino acid metabolism and carbohydrate metabolism are carboxylic acids. The role of acylcarnitines in amino acid and carbohydrate metabolism often receives less attention than their role in fatty acid oxidation, but is of no less importance. Insulin resistance is closely related to carnitine metabolism [6]. Numerous medications and environmental toxins and their metabolites are carboxylic acids. Activated carboxylic acid drugs and activated carboxylic acid metabolites of drugs have been associated with adverse events in humans [7]. Formation of carnitine esters of these metabolites may allow the body to excrete the ester in the urine to remove it from the body.

**Carnitine metabolites**

Carnitine does not appear to be degraded by human cells, but is degraded by fungi, bacteria, and other microbes. As we learn more about the role of microbiota in human homeostasis, we are learning that the role of microbiota in carnitine metabolism is also important, to the point of perhaps having clinical implications. Some microbes can synthesize carnitine from very simple precursors, and others can degrade it to the point that carnitine can be their only carbon source. Thus, our gut microbiota may be a source of carnitine in addition to the carnitine actually consumed in the diet, or our gut microbiota may degrade carnitine consumed in the diet, reducing the carnitine available for absorption by the enterocytes. The metabolites in the carnitine degradative pathways of microbes may be released into the gastrointestinal lumen and absorbed by our enterocytes. These microbial metabolites can then be transported in the bloodstream to an organ such as the liver for further metabolism. Such a sequence of events may be relevant to a recent study that resulted in many statements in the public press suggesting that regular consumption of foods containing high levels of carnitine is harmful to cardiovascular health [8]. However, a few weeks later a review was published suggesting a role for carnitine in the secondary
prevention of cardiovascular disease [9]. Quaternary amines such as carnitine and choline [10] are converted to trimethylamine by some bacteria. Trimethylamine is converted to trimethylamine-N-oxide (TMAO), which has been recognized as toxic. Red meat is an excellent source of carnitine, and the study showed that individuals who regularly eat red meat have an increase in their plasma TMAO concentrations after eating a very large amount of red meat at one sitting. However, this did not occur when individuals who had not eaten meat for a long time also ate a large amount of red meat at one sitting. These data are consistent with the hypothesis that the regular meat-eating individuals have a gut microbiota including microbes that readily convert carnitine to trimethylamine, and the gut microbiota of individuals who do not eat meat does not include microbes that readily convert carnitine to trimethylamine.

Although it has been recognized for many years that bacteria in the gut of rats and of people are responsible for the fishy smell observed in some individuals [11], most carnitine research has not included the role of the gut microbiota in the design of experiments. This can no longer continue.

**Interorgan carnitine homeostasis and fuel homeostasis**

Liver is considered the hub for much of metabolism. This is also true for carnitine metabolism. The liver is a major site of carnitine biosynthesis, and it has many pathways involving carboxylic acids, which means that carnitine has many potential roles in liver homeostasis. Bile is produced by the liver in peroxisomes and provides the body with a mechanism to excrete compounds into the intestinal lumen that can improve fat digestion, but also provides a mechanism for compounds to be excreted in the feces (for example, bilirubin) that cannot be easily excreted via the urinary route. It has been known for quite a long time that bile contains acylcarnitines [12].

The kidney is another site of carnitine biosynthesis in humans. An additional very important role is that clearance of acylcarnitine species by the kidney provides the body with a critical way to eliminate unneeded or toxic carboxylic acids from the body.

Neither skeletal muscle nor cardiac muscle can synthesize carnitine, but requires it for many aspects of metabolism. Many papers have been published concerning the importance of carnitine in muscle during different levels of exercise. Human skeletal muscle has very high concentrations of stored carnitine. Cardiac muscle with its continuous contraction uses lipid-based fuel substrates preferentially with the accompanying requirement for carnitine. Although in liver, medium-chain fatty acids can be transported into the mitochondria for beta-oxidation without
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Carnitine; carnitine is required for transport of medium-chain fatty acids into muscle mitochondria for beta-oxidation. It is interesting that liver and muscle also have different isoenzymes of carnitine palmitoyltransferase with somewhat different regulatory characteristics.

Brain is traditionally considered a glucose-using organ with ketones providing a significant amount of energy during starvation. However, carnitine and particularly acetylcarnitine are found in the brain and have been proposed as useful therapeutic agents during disease. We are just beginning to understand the many roles of carnitine in the brain [13]. A brain-specific carnitine palmitoyltransferase has been identified and characterized in brain [14]. Although this homologue of the carnitine acyltransferase family has a sequence very similar to those found in the liver and muscle, it does not appear to be involved in fatty acid oxidation, but rather involved in redox homeostasis of the brain.

Conclusions

What we think we know

Carnitine is clearly important in oxidation of fatty acids, transport of carboxylic metabolites to the site where they are needed, removal of carboxylic compounds that are not needed or are toxic, and release of coenzyme A whose concentration may have become limiting.

What we think might be (exciting hypotheses)

Carnitine under most physiological conditions is a zwitterion with a hydroxyl group and a carboxylic group that are potential reactive sites, which could lead to a long list of metabolites. This chapter has focused only on esterification with activated carboxylic acids. Although human cells do not degrade carnitine, our gut microbiota do, which adds to the list of metabolites.

The lists of acylcarnitines that have been published from global metabolomic studies in mammals are now in the hundreds. Equally interesting is that so many metabolic studies that compare one physiological state to another physiological state in both animals and humans find that one or more acylcarnitines are among the list that are the most different between the two states. It is also noteworthy that the ability to identify a couple dozen acylcarnitines in blood spots has enabled newborn screening programs to diagnose a large number of diseases with a wide variety of symptoms. Whether carnitine is a modulator or only an indicator of metabolism in these many different physiological states, it is clear that it is involved in many aspects of human homeostasis and is a fertile area to explore the myriad hypotheses that come to mind when perusing the
long list of biological acylcarnitines published in the literature over the past decade.

References
Introduction

L-Carnitine (β-hydroxy-γ-trimethyl-amino-butyric acid) is a conditionally essential nutrient that was discovered more than a century ago (Bieber 1988). It is widely distributed in biological tissues of all mammals and performs several functions in addition to its essential role in membrane trafficking of fatty acids and facilitating long-chain fatty acid oxidation (Fritz 1963). In an average 70-kg adult, the total amount of carnitine is estimated to be around 100 mmol (=16 g) but can vary depending on the muscle mass, which contains approximately 98% of this amount. Only 1.6% of the L-carnitine is found in the liver and kidneys, with the remainder found in the plasma and all other tissues (Engel and Rebouche 1984). These facts
profundely emphasize the critical role of carnitine in the physiology of cardiac and skeletal muscle.

In humans, fatty acids represent the primary source of energy for the heart, as well as the major energy substrate for oxidative skeletal muscles in the postabsorptive stage and during rest and mild-intensity exercise (van Hall et al. 2002, Lopaschuk et al. 2010). In the heart, oxidation of fatty acids (β-oxidation) in mitochondria accounts for 50–70% of ATP production. Glucose, on the other hand, provides most of the remaining energy requirements of the muscle, and a state of competition governs the cellular selection between the two substrates (Lopaschuk et al. 2010). As stated earlier, carnitine is essential for fatty acid oxidation, which makes it an attractive target for modulating energy metabolism, especially in tissues relying heavily on fatty acids for energy production, such as the cardiac and skeletal muscles. Interestingly, carnitine can also regulate glucose metabolism, mainly by indirectly modulating the activity of pyruvate dehydrogenase enzyme complex (PDC), the enzyme controlling glucose oxidation (which will be discussed later).

While the classic and crucial role of carnitine in the admittance of long-chain fatty acyl coenzyme A (CoA) to the mitochondria for β-oxidation is well established, its roles in fuel selection, metabolic flexibility, and insulin resistance have only recently received more attention (Koves et al. 2008, Noland et al. 2009, Muoio et al. 2012, Schroeder et al. 2012). These functions are performed through the integrated actions of carnitine acyltransferase and transfer proteins that lead to the modulation of cellular CoA pools and shifting fatty acyl groups (as acylcarnitine) away from key enzymatic and cell signaling processes that otherwise could be affected by these metabolites (Ramsay and Zammit 2004). Therefore, carnitine is actively involved in the regulation of metabolic processes in the cell including fuel sensing, fuel selection, and fuel metabolism. Current animal and human research is aimed at clarifying the role of the carnitine system in different biological tissues motivated by the ongoing quest for metabolic therapy for cardiovascular disease, diabetes, obesity, and metabolic syndromes. Since the majority of carnitine content is concentrated in muscles, along with the fact that carnitine is crucial to metabolic functions, this chapter will focus primarily on the metabolism and function of the carnitine system in skeletal and cardiac muscles.

**Carnitine homeostasis**

In humans, carnitine homeostasis is maintained through its dietary intake, carrier-mediated absorption and uptake into tissues, de novo biosynthesis, and renal reabsorption. Daily carnitine requirements are variable depending on the metabolic rates and body mass, but average around 5 µmol/day/kg of body weight (= 0.8 mg/day/kg) (Vaz and Wanders
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Dietary intake of carnitine represents a primary source of this vital substance. Animal dietary sources, mainly red meats, are generally rich in carnitine. However, it is also found in vegetables, grains, and fruits, although in smaller amounts (Steiber et al. 2004). Adults obtain approximately 75% of their carnitine requirements from dietary intake and 25% by endogenous biosynthesis (Vaz and Wanders 2002). Carnitine is readily absorbed by healthy intestines, through both carrier-mediated transport and passive diffusion, to maintain nearly constant plasma concentrations of total carnitine (49–50 µM), free carnitine (38–44 µM), and acetylcarnitine (6–7 µM) (Evans and Fornasini 2003, Reuter et al. 2008). Plasma levels and urinary excretion of carnitine positively correlate with nutritional intake in lean individuals (Steiber et al. 2004). In addition, males tend to have more plasma and urine carnitine concentrations than females (Steiber et al. 2004, Reuter et al. 2008, Lombard et al. 1989).

The bioavailability of dietary carnitine is much higher than that of supplemental doses (Evans and Fornasini 2003), emphasizing the importance of its natural dietary sources and indicating a saturable carrier-mediated absorption in the gastrointestinal tract (Matsuda et al. 1998). In fact, vegetarians, due to lower carnitine content in their diet, have less plasma carnitine levels and urinary excretion than omnivores, although with no apparent clinical significance (Lombard et al. 1989). Carnitine intestinal absorption is characterized by slow uptake, prolonged retention in intestinal mucosa, and slow transport to the blood. The baseline renal clearance of carnitine is 1–3 ml/min after a very efficient, yet saturable, tubular reabsorption of 98–99% efficiency (Evans and Fornasini 2003).

Carnitine biosynthesis mainly occurs in liver and kidney, although the brain also has a small potential for its production. The original precursors of carnitine are lysine and methionine. However, the first metabolite in the carnitine biosynthesis pathway is 6-N-trimethyllysine, and the direct precursor is γ-butyrobetaine (Vaz and Wanders 2002). The process requires vitamin C, pyridoxine (vitamin B₆), niacin (vitamin B₃), and iron as necessary cofactors (Steiber et al. 2004). Recently, several carnitine biosynthesis enzymes as well as uptake proteins were shown to be under transcriptional control of the PPARα transcription factor, including 4-N-trimethylaminobutyraldehyde dehydrogenase and the plasmaemmal organic cation Na⁺-dependent transporter 2 (OCTN2) (van Vlies et al. 2007, Wen et al. 2012). PPARα is an important transcriptional factor regulating many components of cellular energy metabolism, including fatty acid uptake and oxidation (Mandard et al. 2004, Lopaschuk et al. 2010). This further highlights the relationship between carnitine and its functions in fatty acid metabolism.
Carnitine system components and associated carnitine insufficiency

The large carnitine content in heart and skeletal muscle, despite the inability of these tissues to synthesize carnitine, requires a high-efficiency muscular extraction of carnitine from the blood against a high concentration gradient. This occurs via a plasmalemmal transporter, the organic cation Na\(^+\)-dependent transporter 2 (OCTN2) (Tamai et al. 1998). This high-affinity transporter concentrates carnitine inside the cardiomyocyte to millimolar levels (Idell-Wenger et al. 1978), despite the fact that plasma levels are only in the 50 micromolar range (Reuter et al. 2008). Expectedly, mutational defects of OCTN2 cause primary systemic carnitine deficiency. The affected patients show hypoketotic hypoglycemia and muscle weakness that develop over time into skeletal myopathy, hepatic steatosis, male infertility, and cardiomyopathy (Vaz et al. 1999, Tamai 2012). In addition, several drugs that affect OCTN2 uptake of carnitine, including, for example, xenobiotics, can interfere with carnitine intestinal absorption and tissue distribution, resulting in the induction of a secondary carnitine deficiency (Tamai 2012). Insulin, on the other hand, can increase carnitine content in skeletal muscles after 5 hours of co-administration with carnitine, an effect ascribed to insulin-mediated stimulation of sarcolemmal Na\(^+\)/K\(^+\) ATPase and an increase in OCTN2 expression (Stephens et al. 2006). A recent study on carnitine uptake in skeletal muscle showed that muscular contraction increased carnitine uptake mostly by increasing OCTN2 translocation to the sarcolemmal membrane (Furuichi et al. 2012), suggesting a link between carnitine uptake and availability in the muscle to processes requiring high ATP production (i.e., contraction).

The reversible transformation of free carnitine to acylcarnitine, and hence the localization or transport of acyl moieties among intracellular compartments, is dependent on the activities of several transferase enzymes that are widely and unevenly distributed over the cytosol and cellular organelles. These distinct proteins are members of the carnitine acyltransferase family, which play central and integrative roles in cell metabolism (Figure 2.1). Each of these enzymes shows properties that fit the specific reaction it performs in terms of its localization, the length of acyl group it transfers, and the regulatory mechanism of its activity. The essential role of carnitine acyltransferases in cellular metabolism is strongly evident in clinical pathologies originating from defects in these enzymes. The clinical picture of these defects may vary from muscle weakness, seizures, and heart failure due to mild deficiencies of these enzymes, to death in more serious deficiencies (Bartlett and Pourfarzam 2002).

Carnitine palmitoyltransferase I (CPT I) is one important member of the carnitine acyltransferase family. It is associated with the outer mitochondrial membrane with its catalytic site in direct contact with cytosolic
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long-chain acyl CoA. It specifically transfers long acyl chains to carnitine to form long-chain acylcarnitines, thereby allowing long-chain acyl group flux into the mitochondrial matrix via the carnitine acylcarnitine translocase (CACT) protein, which spans the inner mitochondrial membrane that is otherwise impermeable to such metabolites (Zammit 1999). Therefore, CPT I can be viewed as catalyzing the first and rate-limiting step in mitochondrial β-oxidation by facilitating fatty acyl group import into the mitochondria to be oxidized. Importantly, this enzyme is susceptible to potent allosteric inhibition by malonyl CoA, which is produced by acetyl CoA carboxylase (ACC) and degraded by malonyl CoA decarboxylase (MCD) (Paulson et al. 1984, Dyck and Lopaschuk 2002). Consequently, CPT I is a major regulation site of fatty acid oxidation. In addition to the mitochondria, CPT activity is also found in peroxisomes and endoplasmic reticulum, although there is ambiguity about the identity of the responsible enzyme(s) (Ramsay and Zammit 2004). CPT I also exists in

Figure 2.1 A schematic view of the mitochondrial carnitine system. Carnitine is the common substrate for all components of this system. The carnitine system is involved in several metabolic functions of the cell including fatty acid flux into the mitochondria, modulation of coenzyme A (CoA) pools, and efflux of acylcarnitines out of mitochondria and cells into the extracellular fluid. CACT, carnitine acylcarnitine translocase; FACS, fatty acyl CoA synthase; CAT, carnitine acetyltransferase; CPT I and CPT II, carnitine palmitoyltransferases I and II, respectively; TAG, triacylglycerol.
three genetically separate isoforms expressed in different tissues: CPT IA expressed mainly in liver and kidney (also known as L-CPT I), CPT IB predominantly expressed in cardiac and skeletal muscle (also known as M-CPT I), and CPT IC found in endoplasmic reticulum of neurons in the brain (Sierra et al. 2008, Zammit et al. 2009). Little is known about the third isoform. Only few known mutations of CPT I are compatible with life, and all occur in the liver-type enzyme. These are associated with reduced activity, lower fatty acid β-oxidation, fasting intolerance, and high lethality (Zammit et al. 2009). As in humans, homozygous deficiency of CPT IB is lethal in mice (Invernizzi et al. 2001, Ji et al. 2008). However, a recent study using heterozygous CPT IB knockout mice (CPT IB$^{+/−}$), which show deficiency of this enzyme, investigated the effects of CPT IB deficiency in pressure overload cardiac hypertrophy (He et al. 2012). Death from congestive heart failure and hypertrophy occurred when these animals were challenged with severe pressure overload, although they showed normal cardiac heart structure and function under the basal condition. These animals also developed exacerbated cardiac hypertrophy, functional impairment, and cardiac remodeling when subjected to milder degrees of pressure overload. Importantly, these CPT IB-deficient mice displayed overt mitochondrial abnormalities and accumulation of myocardial lipid intermediates including triacylglycerol and ceramides, and displayed higher cardiomyocytes apoptosis (He et al. 2012).

Both esterified carnitine (such as acylcarnitines) and free carnitine gain access to the mitochondrial matrix via CACT within the inner mitochondrial membrane (Ramsay and Tubbs 1975, Indiveri et al. 1990). This carnitine carrier exchanges carnitine for acylcarnitines of variable chain lengths ranging from 2 to 18 carbons, with higher affinity toward longer chains and a propensity to transport these long-chain acylcarnitines into the mitochondrial matrix by virtue of a hydrophobic binding site for the interaction with the acyl moiety (Indiveri et al. 2011, Tonazzi et al. 2012). CACT also exists in membranes of peroxisomes to facilitate import and export of fatty acid oxidation metabolites (Fraser and Zammit 1999). Many mutations in the gene coding for CACT have been identified leading to “carnitine carrier deficiency,” which is characterized by nonketotic hypoglycemia, hyperammonemia, serious fasting-induced comas, liver disease, muscle weakness, and seizures (Magoulas and El-Hattab 2012). Usually patients show increased plasma levels of long-chain acylcarnitines with lower plasma-free carnitine (Rubio-Gozalbo et al. 2004, Indiveri et al. 2011).

Another carnitine acyltransferase that complements the activity of CPT I is carnitine palmitoyltransferase II (CPT II). It is bound to the inner mitochondrial membrane with its catalytic site facing the matrix. Its main function is to transfer the acyl group from acylcarnitine to mitochondrial-free CoA to regenerate long-chain acyl CoA that is later handled by β-oxidation enzymes (Zammit 1999). However, it has been proposed that in the case of
surplus long-chain acyl CoA delivery to the mitochondrial matrix (which could occur in states of decreased demand on ATP or mitochondrial over-load), CPT II activity shifts to the opposite direction, thereby producing long- and medium-chain acylcarnitines in an attempt to export excess acyl groups out of the mitochondria (Koves et al. 2008). Interestingly, CPT I and CPT II coexist across contact sites between the two mitochondrial membranes (Fraser and Zammit 1998), emphasizing the close functional relationship between these two enzymes. Many CPT II mutations have been described, with severe mutations resulting in death in infancy, and milder cases presenting with muscle fatigue after exercise or with starvation. CPT II defects may lead to myopathy and, in some mutations, to insulin resistance (Haap et al. 2002, Isackson et al. 2008). CPT II defects can share similar phenotypes with defects in CACT, including the development of nonketotic hypoglycemia (Rubio-Gozalbo et al. 2004).

A third member of the carnitine system is carnitine acetyltransferase (CAT). It is structurally and functionally related to the other carnitine acyltransferases with specificity toward short-chain acyl groups (Jogl and Tong 2003, Cordente et al. 2006). Although initially identified early, it is the least characterized of the carnitine acyltransferases. It is localized in the mitochondrial matrix and peroxisomes, but has also been found in the endoplasmic reticulum and nucleus (Ramsay and Zammit 2004). In peroxisomes, it is thought to assist in the exporting of short-chain acyl groups (e.g., acetyl and propionyl groups produced by peroxisomal fatty acid oxidation) to the mitochondria, by transferring these acyl groups from a CoA ester to a carnitine ester. Moreover, it may provide an alternative means of exporting acetyl moieties, in addition to the acetate form (produced by peroxisomal acyl CoA thioesterases), to the cytosol, which may eventually support malonyl CoA production (Westin et al. 2008). Mitochondrial and peroxisomal CAT are encoded by the same gene, with alternative splicing resulting in different variants targeted to either subcellular organelle (Corti et al. 1994).

To our knowledge, only two cases of CAT insufficiency have been reported in the literature. The first case was reported in 1979, and involved an infant who died of ataxic encephalopathy with serious neurological symptoms and liver dysfunction (DiDonato et al. 1979). Interestingly, pyruvate oxidation was also disrupted secondary to insufficient activity of a deficient CAT in the multiple tissues tested (DiDonato et al. 1979). This suggests an important relationship between CAT and pyruvate dehydrogenase activity (as will be described later). The second CAT-defective case was described in 1999, and involved a female infant who died at 1 year of age with cerebral and muscular symptoms (Melegh et al. 1999). In addition to the overt neurological abnormalities, structural and functional abnormalities of the mitochondria of skeletal and cardiac muscle were detected (Melegh et al. 1999). Recently, CAT has been increasingly being
viewed as a key component of energy metabolic regulation in the heart and skeletal muscle and seems to be actively involved in energy substrate selection, insulin sensitivity, and carbohydrate metabolism.

Carnitine octanoyltransferase (COT) represents the last known member of the carnitine acyltransferase enzyme family. It is assumed to exist only in peroxisomes and preferentially interacts with medium-chain acyl CoA esters. Similar to CAT, it is assumed to provide a mechanism through which acyl moieties are transported out of the peroxisomal core (Ramsay and Zammit 2004).

In summary, many carnitine system disorders have been discovered to date, all of which result in lipid metabolic disturbances secondary to impairments of fatty acid trafficking in the cell. In many cases of carnitine insufficiency, exogenous carnitine administration can be a life-saving medication.

Carnitine metabolic effects on cardiac and skeletal muscle

In addition to the classic and extensively studied role of the carnitine system in allowing entry of long-chain acyl CoAs into the mitochondria, several other metabolic functions of the carnitine carrier system have been suggested in cardiac and skeletal muscle, including the following:

A role for CAT in acetyl CoA buffering: Implications in cardiac metabolic remodeling

By transferring acyl groups back and forth between carnitine and CoA, carnitine acyltransferases have the potential to modulate acyl CoA/CoA ratios in the cytosol and the mitochondrial matrix. This has consequences in the regulation of fatty acid metabolism, as well as glucose metabolism. To understand this concept, one must first understand the reciprocal inhibitory relationship between glucose and fatty acid oxidation that is known as the Randle cycle (Randle et al. 1963). According to this cycle, fatty acid oxidation can inhibit glucose oxidation through at least two mechanisms. First, cytosolic phosphofructokinase is under inhibitory control by citrate produced by the tricarboxylic acid (TCA) cycle, which is stimulated by acetyl CoA produced by fatty acid β-oxidation. Second, the pyruvate dehydrogenase complex (PDC) is inhibited by an increase in the acetyl CoA/CoA ratio, both by product inhibition (acetyl CoA being a product of PDC) and through stimulating the inhibitory phosphorylation by pyruvate dehydrogenase kinase (PDK) (Lopaschuk et al. 2010). Therefore, fatty acids and glucose compete for the production of ATP. In this context, it is worth mentioning that although fatty acid oxidation has a greater ability to produce ATP than glucose, this comes at the expense of
using more oxygen. Accordingly, fatty acids are considered less efficient as energy substrates than glucose, and a great reliance on their oxidation can decrease mechanical efficiency in the heart (Lopaschuk et al. 2010).

The interplay between carnitine and CoA pools and the modulation of the acetyl CoA/CoA ratio in myocytes has been supported by studies using carnitine supplementation to isolated mitochondria, myocytes, whole heart, or skeletal muscles or through modulating the expression of CAT (Pearson and Tubbs 1967, Lysiak et al. 1988, Broderick et al. 1992, 1993, 1995a, 1995b, Saddik et al. 1993, Koves et al. 2008, Muoio et al. 2012, Schroeder et al. 2012). It is now widely accepted that CAT plays a key role in the modulation of the mitochondrial acetyl CoA/CoA ratio. CAT is believed to act as a buffer system preventing the accumulation of acetyl CoA that can inhibit PDC activity and pyruvate (or glucose) oxidation (Figure 2.2). Furthermore, the continuous regeneration and availability of free CoA is essential for supporting many mitochondrial enzymatic processes (Zammit et al. 2009). To emphasize a role for CAT in acetyl CoA buffering, a recent study used a hyperpolarized $^{13}$C magnetic resonance technique to determine the immediate fate of infused $^{2–13}$C pyruvate in rat hearts (Schroeder et al. 2012). This study suggested that half of the PDC-derived acetyl CoA quickly (within 50 seconds) cycles through CAT into the acetylcarnitine pool prior to reconversion to acetyl CoA and incorporation into the Krebs cycle. Moreover, dichloroacetate (DCA) (an inhibitor of PDK) and dobutamine (a $\beta$-receptor agonist) accelerated and decelerated, respectively, the rate of acetylcarnitine accumulation in the heart. This indicates that the acetylcarnitine pool acts like a storage pool that can accommodate any excess in acetyl CoA production by PDC (which occurs following DCA administration), and can also quickly provide more acetyl CoA to the TCA cycle whenever needed (such as following dobutamine administration) all through the dynamic action of CAT (Schroeder et al. 2012).

This important regulatory action of CAT on myocardial glucose oxidation was shown by early experimental studies conducted by us in isolated working rat hearts. Carnitine loading to normal, ischemic, carnitine-deficient, and diabetic rat hearts increased glucose oxidation at the expense of fatty acid oxidation, and improved contractile function in those hearts (Broderick et al. 1992, 1993, 1995a, 1995b). This appears to contradict the fact that carnitine is a substrate of CPT I, the first enzyme responsible for fatty acid uptake into the mitochondria. Nonetheless, this peculiar behavior can be explained by the concept of equilibrium between free CoA and acetyl CoA, and the latter’s regulation of PDC activity. Being also a substrate of CAT, high free carnitine levels in mitochondria would stimulate CAT-mediated transfer of acetyl groups from acetyl CoA to carnitine, thus decreasing the acetyl CoA/CoA ratio. As explained above, this would translate into relieving the constituent inhibition of PDC and pyruvate oxidation (Broderick et al. 1992).
Several clinical trials have shown that L-carnitine improves metabolic and functional profiles in the ischemic heart (Kamikawa et al. 1984, Canale et al. 1988, Rizzon et al. 1989, Corbucci and Loche 1993, Tarantini et al. 2006, Xue et al. 2007). These advantages are evident as improved exercise tolerance and ventricular function in angina patients (Kamikawa et al. 1984, Canale et al. 1988), decreased plasma levels of markers of cell injury (Xue et al. 2007), reduced incidence of arrhythmia (Rizzon et al. 1989), and improved recovery and survival after a myocardial infarction.
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(Corbucci and Loche 1993, Tarantini et al. 2006). These beneficial effects could occur as a result of carnitine activation of CAT, resulting in an increase in PDC and pyruvate oxidation (Broderick et al. 1992, 1993, 1995a, 1995b). However, alternative cardioprotective effects of carnitine cannot be excluded. These may include counteracting the toxic effects of accumulated acyl CoAs by either admitting them to the fatty acid β-oxidation pathway or by exporting them out of myocardial cells and eventually into urine as acylcarnitines (see below).

Potential role for CAT in malonyl CoA axis

In addition to affecting PDC activity, we also propose that CAT can simultaneously regulate fatty acid metabolism by a series of catalytic actions inside and outside mitochondria leading to malonyl CoA inhibition of CPT I and β-oxidation. We proposed that in times of low energy demand and surplus activity of β-oxidation, excess acetyl CoA production can exceed the rate at which it is fed into the TCA cycle for subsequent ATP generation (Saddik et al. 1993). Consequently, CAT may facilitate export of excess acetyl groups derived from acetyl CoA out of mitochondria. This is achieved by transferring acetyl groups to carnitine, thus increasing acetylcarnitine concentration, which is then exported to the cytosol. Thereafter, this acetylcarnitine can provide a source for acetyl CoA regeneration by a proposed cytosolic CAT. The subsequent elevated cytosolic acetyl CoA would then drive the production of malonyl CoA through the enzymatic action of ACC (Figure 2.2) (Saddik et al. 1993). Elevated malonyl CoA levels, in turn, confer a potent inhibition of CPT I and fatty acid oxidation (Paulson et al. 1984, Dyck and Lopaschuk 2002, Reszko et al. 2004). Nonetheless, the presence of a cytosolic CAT activity was previously questioned in a study on rat cardiomyocytes by Abbas et al. (1998). Their results attributed only 5–11% of the total cellular activity of CAT to the cytosolic fraction, which is in accordance with recent preliminary data in our lab. This activity was considered insignificant by the authors. In addition, they argued that their estimated rate of acetyl CoA production in the cytosol (only 0.3 nmol/minute/mg protein) would be insufficient to sustain flux of acetyl CoA to the ACC-mediated malonyl CoA production. Consequently, they concluded that CAT is not present in the cytosol, and therefore does not affect malonyl CoA production (Abbas et al. 1998). Conversely, we find this activity to be more than enough to supply the substrate for the production of malonyl CoA bearing in mind the turnover rates of malonyl CoA (Abbas et al. 1998, Reszko et al. 2004) and sensitivity of the predominant ACC isoform in the heart to acetyl CoA concentration (Saddik et al. 1993). Moreover, heart CPT I is extremely sensitive to the smallest change in malonyl CoA concentration. Consequently, small changes in cytosolic acetyl CoA concentration could theoretically lead to
a strong inhibition of CPT I and fatty acid β-oxidation (Saddik et al. 1993). In conclusion, CAT, whether in peroxisomes (Zammit 1999, Ramsay and Zammit 2004) or in the cytosol (Saddik et al. 1993), is a strong candidate for being involved in direct control of β-oxidation through the acetyl CoA-malonyl CoA axis.

ATP-citrate lyase (ACL) is another enzyme proposed to play at least a partial role in the regeneration of acetyl CoA in the cytosol of muscle. Poirier et al. (2002) suggested that ACL uses citrate molecules exported from the mitochondria to the cytosol as its substrate to provide cytosolic acetyl CoA for the production of malonyl CoA by ACC. Nonetheless, they did not exclude a role for CAT and acetylcarnitine as another source (Poirier et al. 2002) (Figure 2.2).

Based on the above discussion, activation of CAT by increasing intracellular carnitine can increase glucose oxidation and is proposed to also inhibit fatty acid oxidation via promoting the production of malonyl CoA. A recent study used an alternative experimental way to specifically increase CAT activity in human skeletal myocytes (Noland et al. 2009). The researchers transfected skeletal myocytes with recombinant adenoviruses overexpressing CAT. These cells showed increased glucose uptake and attenuated lipid-induced suppression of glucose oxidation. Addition of carnitine further enhanced those effects (Noland et al. 2009). A more recent study highlighted CAT important effects by using muscle-specific CAT deletion in mice (Muoio et al. 2012), which will be discussed in the context of insulin sensitivity below.

**Carnitine in insulin resistance and metabolic inflexibility**

Insulin resistance is a hallmark of type 2 diabetes mellitus and obesity. Currently, lipotoxicity is a widely accepted theory explaining the induction of insulin resistance (Morino et al. 2006, Holland et al. 2007, Muoio and Koves 2007, Bosma et al. 2012). This paradigm proposes that excess lipid intermediates in the cytosol, including ceramides, long-chain acyl CoAs, gangliosides, or diacylglycerol, can interfere with and inhibit insulin signaling in insulin-sensitive tissues (such as skeletal muscle) due to their nature as signaling molecules and constituents of cell membranes (Morino et al. 2006, Holland et al. 2007, Muoio and Koves 2007, Bosma et al. 2012). For example, pharmacological inhibition of CPT I with etomoxir leads to accumulation of intramyocellular lipid and increased insulin resistance possibly through interference with insulin signaling (Dobbins et al. 2001). Likewise, elevated malonyl CoA inhibits CPT I and has the potential to result in decreased fatty acid oxidation rates as well as accumulation of cytosolic long-chain fatty acids, leading to insulin resistance (Ruderman et al. 1999). A related observation is that obese patients with insulin resistance have low CACT expression and activity in skeletal muscles (Peluso
et al. 2002). This can be correlated with the accumulated intramuscular lipids found in these patients and might support the assumption that decreasing mitochondrial uptake and oxidation results in lipotoxicity and insulin resistance.

Metabolic inflexibility is a related concept that refers to the impaired ability of the cell to switch from the use of fatty acids to glucose for energy production in response to metabolic cues (Kelley and Mandarino 2000). In a recent study, the role of CAT was investigated in insulin resistance and metabolic inflexibility using CAT knockout mice, CAT knockdown and overexpressing human skeletal myocytes, and diabetic and obese human subjects on carnitine supplementation (Muoio et al. 2012). CAT gene deletion resulted in glucose intolerance and insulin resistance and impaired the normal postprandial metabolic switch to glucose. Here again, CAT is shown to act as an outlet for acetyl CoA in states of superfluity (as in transition from fasting to feeding). Loss of CAT function raised cardiac and skeletal muscle long-chain acylcarnitine levels and blunted the carnitine-mediated stimulation of PDC activity and the pyruvate competition with fatty acids as a fuel. Interestingly, the study also found a severely depressed CAT mRNA expression in patients with type 2 DM. Glucose tolerance was improved upon carnitine supplementation in diabetic patients, whereas CAT expression level in isolated skeletal muscles was directly correlated to glucose uptake, fuel selection, and acetylcarnitine efflux (Muoio et al. 2012).

Contrary to the lipotoxicity model, Muoio et al. proposed a scenario where high fatty acid β-oxidation rates in mitochondria overloaded with acyl CoAs (as seen in obesity, diabetes, and a high-fat diet) may outpace the TCA cycle, thereby causing incomplete oxidation of these acyl CoAs and leading to the esterification of carnitine and accumulation of acylcarnitines that block insulin sensitivity (Muoio and Koves 2007, Koves et al. 2008) (Figure 2.3). The genetic inhibition of mitochondrial fatty acid β-oxidation through deletion of MCD (higher malonyl CoA levels) protected against diet-induced glucose intolerance despite high intramuscular levels of long-chain acyl CoAs (Koves et al. 2008). In accordance with these findings in animal models, acylcarnitines have also been implicated in insulin resistance in humans (Hoppel and Genuth 1980, Soeters et al. 2009, Michalik et al. 2010).

Several studies have reported diminished plasma carnitine levels in type 1 and 2 diabetics (Tamamogullari et al. 1999, Mamoulakis et al. 2004, Kilicli et al. 2010). Patients with type 2 diabetes showed ~25% lower carnitine concentrations, especially with late complications (Tamamogullari et al. 1999, Poorabbas et al. 2007). The exact reason why carnitine is low with diabetes is unknown. However, Noland et al. found a reduced hepatic mRNA of carnitine biosynthetic enzymes (Noland et al. 2009). Furthermore, carnitine muscle uptake is stimulated by insulin (Stephens et al. 2006). Consequently, insulin deficiency or resistance might explain the decreased carnitine levels
Figure 2.3 Induction of insulin resistance as viewed by lipotoxicity versus mitochondrial overload theories and role of carnitine acetyltransferase (CAT) in metabolic flexibility. According to the lipotoxicity model proposed by some studies, conditions leading to low fatty acid oxidation lead to a building up of cytosolic long-chain acyl CoAs that in turn results in the accumulation of lipid metabolites (e.g., ceramides, long-chain acyl CoA, gangliosides, or diacylglycerol), which can interfere with insulin signaling and induce insulin resistance. In contrast, other studies implicate a state of mitochondrial overload and increased incomplete fatty acid oxidation that outpaces the TCA cycle and causes the production and accumulation of acylcarnitines. By shifting acetyl CoA away from the pyruvate dehydrogenase complex (PDC) and forming acetylcarnitine to be exported from mitochondria and out of cells, CAT indirectly activates PDC and increases the sensitivity toward fuel switch (metabolic flexibility), which is disturbed in carnitine insufficiency accompanying conditions of insulin resistance. CACT, carnitine acylcarnitine translocase; CPT I and CPT II, carnitine palmitoyltransferases I and II, respectively; FACS, fatty acyl CoA synthase; FAT/CD36, fatty acid translocase; GLUT1/4, glucose transporters 1 and 4; IR, insulin receptor; MCT9, monocarboxylate transporter 9; MPC, mitochondrial pyruvate carrier; OCTN2, organic cation Na⁺-dependent transporter 2; TCA, tricarboxylic acid.
in diabetes. Additionally, the lipid overload usually accompanying type 2 diabetes can induce higher long-chain acylcarnitine production and plasma levels (Mihalik et al. 2010), and thus reduces the proportion of free carnitine. Respiratory exchange ratios are also lower in these patients, indicating relatively persistent fatty acid oxidation (metabolic inflexibility) and lipid mitochondrial flux (Kelley 2005, Mihalik et al. 2010).

Carnitine insufficiency is also found in animals with insulin resistance states such as aging, obesity, and a high-fat diet (Noland et al. 2009). This carnitine shortage is suggested to play a part in mitochondrial dysfunction including elevated incomplete fatty acid $\beta$-oxidation, accumulation of long-chain acylcarnitines, and metabolic inflexibility. Carnitine supplementation reverses mitochondrial abnormalities in these animals, increases tissue efflux and urinary excretion of acylcarnitines, and improves glucose tolerance. In addition, overexpression of CAT in human skeletal myotubes increases glucose oxidation rates and has a salutary effect on metabolic flexibility and insulin sensitivity (Noland et al. 2009). Similarly, exogenous carnitine improves insulin sensitivity in mice with genetic diabetes (Power et al. 2007).

Whatever the source of long-chain acyl groups is (cytosolic long-chain acyl CoAs or the mitochondrial long-chain acylcarnitines), these metabolites seem to be deleterious to insulin sensitivity. Therefore, supplementation of free or short-chain carnitine can potentially provide a carrier for these acyl groups and a means for their mitochondrial uptake and oxidation or for their efflux out of the cell, through the bloodstream and into urine. Another benefit of carnitine esterification to long-chain acyl groups by CPT enzymes is that, as with acetylcarnitine, it can reproduce free CoA and thereby allow the continuation of fatty acid oxidation and other CoA-dependent processes (Lopaschuk et al. 1994, Ramsay et al. 2001). This principle of acylcarnitine export is supported by the fact that acylcarnitines are measurable in plasma and reflect the cytosolic acylcarnitine pool. Additionally, perturbations of the distribution of L-carnitine into different chain length acylcarnitines within the total carnitine pool are indicative of mitochondria dysfunction and help in diagnosing some defects and disorders of fatty acid oxidation (Wanders et al. 1999, Zammit et al. 2009, Kilicli et al. 2010). Similarly, nutritional and metabolic status including diet composition, fasting, and insulin resistance can all affect plasma acylcarnitine profile (Costa et al. 1999, Tamamogullari et al. 1999, Koves et al. 2008, Kien et al. 2011).

The plasmalemmal transporter exporting acylcarnitines has yet to be identified, although the monocarboxylate transporter 9 (SLC16A9, also known as MCT9) is a potential candidate (Suhre et al. 2011). However, some of the acylcarnitines (especially those of long chains) might not exit the cell if they blend with other amphipathic lipids in cell membranes (Ho et al. 2002). This might provide an attractive explanation for the interference
with insulin signaling by lipid intermediate proximally at the cell membrane level (Holland et al. 2007).

Finally, it is not clear whether insulin resistance in skeletal and cardiac muscle is a result of cytosolic accumulation of lipid intermediates secondary to decreased fatty acid oxidation, or if it is actually induced by an accelerated, but incomplete, mitochondrial fatty acid oxidation in response to states of mitochondrial overload with the excess production and efflux of acylcarnitine. In either case, a state of carnitine deficiency (especially the free form) and a potential insufficiency of at least CPT, CACT, and CAT seem to be either causing or accompanying this metabolic syndrome, and that supplementation with L-carnitine should be beneficial. Further studies are needed to explore the interplay between the carnitine system and insulin sensitivity.

Conclusion

Carnitine has been long known to be actively involved in cardiac and skeletal muscle metabolism by virtue of its role in intracellular trafficking of fatty acid. However, other potentially important actions of carnitine have emerged, including its effects on modulating CoA pools and the consequences of this on remodeling of energy metabolism, particularly in the heart. Of importance in these effects is CAT and its interplay with PDC through fine-tuning of the mitochondrial acetyl CoA/CoA ratio and the resulting de-inhibition of PDC. This was shown to overcome the inhibitory effect of fatty acid oxidation on glucose oxidation, and thus results in enhanced glucose uptake and oxidation at the expense of fatty acid oxidation, which correlates with an improvement in switching to glucose in response to refeeding. Carnitine supplementation in cardiac pathologies, although requiring long periods of time to achieve therapeutic efficacy, was shown by experimental as well as clinical studies to improve cardiac function and help reduce injury. Additionally, carnitine therapy in obesity-induced insulin resistance and diabetes seems to have potential as a therapy. The improvement of insulin sensitivity and glucose tolerance is again ascribed to carnitine stimulatory effects on CAT, as well as on intracellular acylcarnitine pools. In this context, enhancement of full oxidation of long-chain fatty acids (as opposed to incomplete oxidation) along with efflux of long-chain acylcarnitines may prevent mitochondrial dysfunction as well as prevent the interference with insulin signaling.

Acknowledgments

GDL is an Alberta Heritage Foundation for Medical Research scientist. This research was funded by grants to GDL from the Canadian Institutes of Health Research.
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section two

Carnitine transport and metabolism in skeletal muscle
Introduction

Carnitine, essentially meaning “flesh” in Latin, was simultaneously discovered in meat extracts by the Russian scientists Gulewitsch and Krimberg and the German researcher Kutscher in the early part of the twentieth century [1, 2]. Around twenty years passed before carnitine was classified as 3-hydroxy-4-N,N,N-trimethylaminobutyric acid, a water-soluble quaternary amine [3]. Midway through the twentieth century interest in carnitine’s role in biology grew following its identification as vitamin Bt, an essential growth factor in Tenebrio molitor beetle larvae [4]. Shortly thereafter, carnitine’s role in energy metabolism began to be elucidated when German researchers showed that pigeon liver homogenates could exchange acyl groups between carnitine and acyl-coenzyme A (CoA) [5]. In the same year Irving B. Fritz demonstrated that the addition of a skeletal muscle homogenate to rat liver sections elevated palmitate oxidation, where Fritz concluded that carnitine was the ingredient within the muscle homogenate responsible for this finding [6]. In the ensuing decades, pioneering work by Fritz, among others, elucidated carnitine’s
metabolic role in lipid metabolism. These researchers concluded that only long-chain acylcarnitine and not long-chain acyl-CoA could permeate the mitochondrial membranes in a variety of mammalian tissues; as such, carnitine, the principal substrate for the carnitine palmitoyl system, played an obligatory role in long-chain fatty acid oxidation [7–10].

While carnitine is ubiquitous in terms of its distribution in various mammalian tissues, it is concentrated within those which have high adenosine triphosphate (ATP) turnover rates, and thus a need for sustained fuel oxidation. Indeed, approximately 95% of the entire carnitine pool in humans is sequestered within skeletal muscle [11]. In this chapter, the role of carnitine in skeletal muscle substrate metabolism will be described and discussed in order to offer an introduction to the subsequent chapters in this text, which will focus on more applied questions pertaining to skeletal muscle carnitine metabolism in various settings/populations.

The role of carnitine in long-chain fatty acid oxidation

Cytosolic long-chain fatty acids require entry to the mitochondria in order to be exposed to the enzymes of the β-oxidation pathway. Fritz and colleagues identified two functionally unique carnitine palmitoyl transferase (CPT) proteins located in or around the mitochondrial membranes [8] that formed a shuttle system whereby long-chain fatty acids (in the form of acylcarnitines) could gain entry to the mitochondria. CPT-1, an 88-KDa transmembrane enzyme found in the outer membrane of the mitochondrion, catalyzes the formation of long-chain acylcarnitine from cytosolic free carnitine and long-chain acyl-CoA [12]. These lipoprotein-like acylcarnitines can then be translocated through the mitochondrial membranes and into the mitochondrial matrix via a carnitine-specific translocase: carnitine acylcarnitine translocase (CACT), a 32-KDa translocase residing in the mitochondrial membranes. Once in the mitochondrial matrix, carnitine palmitoyl transferase 2 (CPT-2), a 71-KDa protein that is localized near the inner mitochondrial membrane, essentially performs the reverse reaction of CPT-1, forming long-chain acyl-CoA and free carnitine [13]. Owing to similarities in their function, CPT-1 and CPT-2 are structurally similar, where both enzymes function identically in vitro and can only be differentiated by the addition of malonyl-CoA. Indeed, a malonyl-CoA binding site is the only differentiating feature of CPT-1 in vitro when the mitochondrial membranes have been disturbed by extraction and freeze thawing [14]. In vivo, CPT-1 also has transmembrane domains, unlike CPT-2, where the majority of CPT-1, including its malonyl-CoA binding site, is exposed to the cytosol where its substrates reside [15].
It is thought that the CPT shuttle system exchanges mitochondrial carnitine and cytosolic long-chain acylcarnitine on a reciprocal basis. Free carnitine is translocated back to the cell cytosol to act as a CPT-1 substrate once more, whereas mitochondrial long-chain acyl-CoA can subsequently be oxidized, where, following a series of reactions within the β-oxidation pathway, long-chain acyl-CoA is cleaved to acetyl-CoA and short-chain acyl-CoA, where the former can then condense with oxaloacetate in the tricarboxylic acid (TCA) cycle, ultimately facilitating anaploresis and the generation of protons and electrons to be used in oxidative phosphorylation. Carnitine and the CPT system’s role in long-chain fatty acid metabolism are depicted schematically in Figure 3.1.

Alterations in the CPT system have also been implicated in metabolic diseases such as insulin resistance, where maximal rates of CPT-1 activity have been positively correlated with skeletal muscle glucose uptake [16]. In this instance, whether CPT-1 activity measured in muscle homogenates more accurately reflects in vivo CPT-1 function or is more broadly a surrogate of mitochondrial density is hard to say. However, in rodent muscle, overexpressing CPT-1 in vivo leads to numerous metabolic adaptations. Indeed, greater CPT-1 activity in intact mitochondria of CPT-1 overexpressing animals was accompanied by greater palmitate oxidation in skeletal muscle strips [17]. These data suggest that a greater abundance and activity of CPT-1 in skeletal muscle may direct long-chain fatty acids

![Figure 3.1](image-url)  
*Figure 3.1* Carnitine and the CPT shuttle system’s role in long-chain fatty acid oxidation.
Carnitine metabolism and human nutrition

toward an oxidative fate rather than storage, which may have important implications in the context of pathophysiological states associated with lipid accumulation in skeletal muscle. Indeed, Bruce and coworkers went on to demonstrate the metabolic impact of overexpressing CPT-1 in the skeletal muscle of high-fat-fed insulin-resistant rodents. In this setting, increased CPT-1 activity and long-chain fatty acid oxidation were associated with reduced muscle lipid moieties including triacylglycerol, diacylglycerol, and ceramide content [18]. Moreover, increased CPT-1 function and reduced muscle lipid content resulted in improved glucose uptake in muscle strips from animals overexpressing CPT-1. Taken together, these experiments suggest that abundance, and perhaps the functionality, of CPT-1 plays an important role in skeletal muscle lipid metabolism.

Like most other physiological processes, the importance of carnitine and the CPT shuttle system is often best appreciated in patient populations and animal models where the system is perturbed in some way. In addition to the mechanistic insights offered by murine models where the CPT-1 enzyme is overexpressed, the availability of carnitine and in particular free carnitine in muscle likely alters the functionality of CPT-1 in vivo. The topic of skeletal muscle carnitine depletion and loading will be addressed in greater detail in Chapter 5. However, some examples will be briefly discussed here to underscore the obligatory role CPT-1 and carnitine availability plays in muscle long-chain fatty acid oxidation, and subsequently the integration of muscle fuel selection.

While uncommon, disturbances in carnitine homeostasis can have a profound impact on whole fatty acid metabolism. Systemic carnitine deficiency in humans is a rare condition that arises due to an autosomal recessive mutation in the gene responsible for encoding the carnitine transporter OCTN2 (organic cation Na\(^+\)-dependent transporter 2) [19]. OCTN2 plays critical roles in transporting carnitine into tissue and, perhaps most importantly, ensures that filtered free carnitine is reabsorbed in the kidney. An incidence of systemic carnitine deficiency of 1 in approximately 67,000 births has recently been reported by a study conducted in Taiwan [20]. Interestingly, inhabitants of the Faroe Islands have a much higher prevalence of systemic carnitine deficiency (~1 in 300), which has been associated with sudden death in adults [21, 22]. Original case reports of patients with systemic carnitine deficiency showed that these patients have a fraction (~20%) of the muscle carnitine of a healthy individual [23, 24]. Perhaps not surprisingly, this was associated with fat accumulation in skeletal muscle, due to a reduced ability to oxidize long-chain fatty acids. As CPT-1 abundance was not reduced by carnitine deficiency, the addition of carnitine to muscle homogenates restored ex vivo fatty acid oxidation [24]. Similarly, in vivo carnitine supplementation of a patient with carnitine deficiency was effective in improving exercise tolerance and reducing muscle lipid accumulation [23, 24].
The juvenile visceral steatotic mouse, a murine model generally used to study neonatal hepatic steatosis, lacks the carnitine transporter OCTN2. As well as severe perturbations in lipid metabolism, these carnitine-deficient animals also exhibit skeletal muscle glycogen depletion, suggesting that carnitine deficiency results in impaired skeletal muscle fatty acid oxidation while subsequently increasing carbohydrate oxidation. However, supplementing these mice with carnitine can restore skeletal muscle glycogen content while preventing the development of hepatic steatosis [25]. Furthermore, pharmacologically induced carnitine depletion mimics many of the metabolic abnormalities associated with carnitine deficiency. Rats treated with the compound mildronate, which blocks carnitine reabsorption in the kidney and inhibits its biosynthesis in the liver, have reduced whole body palmitate oxidation and develop hepatic steatosis [26].

There are limited data describing the impairment of skeletal muscle function in end-stage renal failure patients undergoing hemodialysis. More than 90% of filtered free carnitine is reabsorbed in the kidney by OCTN2 in vivo. However, during hemodialysis blood is filtered through a synthetic membrane that lacks OCTN2. As such, the cumulative effects of prolonged dialysis will likely result in gradual depletion of whole body and, in particular, skeletal muscle carnitine stores. Indeed, following a decade of hemodialysis treatment renal failure patients have been reported to have skeletal muscle carnitine contents of around 1 mmol·kg\(^{-1}\) dry muscle [27], which is a fraction of those typically reported in healthy humans (>20 mmol·kg\(^{-1}\) dry muscle). Given that this represents approximately 5% of the amount of carnitine usually found in skeletal muscle, it is more than likely that prolonged hemodialysis will result in profound perturbations in skeletal muscle and whole body metabolic regulation. Indeed, long-chain fatty acid oxidation is significantly lower in muscle homogenates obtained from patients undergoing hemodialysis [28], underscoring the notion that reduced muscle carnitine content impairs long-chain palmitoyl-carnitine formation and subsequently palmitoyl-CoA delivery to the mitochondria. At a whole body level, this manifests itself in impaired exercise capacity where the \(\text{VO}_2\) peak is ~50% lower in these patients than in age-matched controls [27, 29].

Unlike healthy humans, who do not respond to acute carnitine supplementation per se, carnitine supplementation alone appears to be a viable means of augmenting the muscle carnitine pool in carnitine-deficient patients, which most likely reflects the reduced plasma-to-muscle carnitine ratios in these patients. Further, \(\text{VO}_2\) max is improved in patients on dialysis following carnitine supplementation [30, 31]. Specifically, Siami et al. intravenously infused patients on dialysis with carnitine three times a week for 6 months. This resulted in a significant 4-mmol·kg\(^{-1}\) dry muscle increase in muscle carnitine content in these
patients. Furthermore, as might be expected, increased muscle carnitine availability was accompanied by a concomitant increase in skeletal muscle long-chain fatty acid oxidation [28]. The impact of end-stage renal failure on skeletal muscle carnitine availability and the potential role for carnitine supplementation in this patient group is discussed in detail by Professor Brass (Chapter 6).

The role of carnitine as an acetyl group buffer

While researchers and students familiar with fat and carbohydrate metabolism are no doubt well aware of carnitine’s central role in fatty acid oxidation, carnitine’s less publicized role in carbohydrate metabolism is often underappreciated. Indeed, in skeletal muscle, the free carnitine pool plays an important role in the maintenance of the mitochondrial acetyl-CoA-to-free coenzyme A (CoASH) ratio during periods of high ATP turnover, such as intense contraction. This phenomenon was first studied in the 1960s by Childress and colleagues [32]. These researchers noticed that the blow fly flight muscle was rich in carnitine and the enzyme carnitine acetyl transferase (CAT). Curiously, during flight, the blow fly flight muscle relies almost exclusively on carbohydrate as a fuel source; however, Childress and coworkers noticed that there was rapid accumulation of acetylcarnitine at the onset of flight [32]. In this instance, it would appear that acetylcarnitine formation prevents acetylation of the mitochondrial CoASH pool, where carnitine acts as a storage tank for the overflow of acetyl groups produced during intense muscular contraction. In the ensuing years, carnitine’s role in buffering excess acetyl group production during intense muscular contraction was confirmed in numerous species, such as amphibians [33], and mammals [34, 35], including humans [36–38].

Similar to CPT-1, the pyruvate dehydrogenase (PDH) enzyme complex is considered to be the rate-limiting step of oxidative carbohydrate disposal. The activation of PDH is regulated by specific phosphatases and kinases that can dephosphorylate (activate) and phosphorylate (inactivate) the PDH complex. In addition, these kinases and phosphatases are also the subjects of metabolic regulation, responding to the cellular redox state and calcium levels. In its role as the rate-limiting enzyme of aerobic carbohydrate oxidation, PDH catalyzes the irreversible decarboxylation of pyruvate, forming acetyl-CoA. At rest, the PDH is inhibited by its own product, acetyl-CoA [39, 40], as per the glucose-fatty acid cycle [41]. During exercise, however, PDH activation status and thus acetyl-CoA production from the PDH increases with increasing exercise intensity. Further, muscle acetyl-CoA and acetylcarnitine content concurrently increase during high-intensity exercise [42, 43], where numerous studies have shown that acetylcarnitine accumulation accounts for the near 80% reduction in muscle free carnitine content observed during intense exercise [34, 36–38].
Indeed, it has been postulated that intense muscular contraction and subsequent maximal PDH activity (~1.5–2 mmol·min⁻¹·kg⁻¹ wet muscle) would acetylate the entire mitochondrial CoASH pool within 1 s. As such, the rapid formation of acetylcarnitine plays a paramount role in maintaining CoASH-consuming reactions during intense contraction. By way of example, approximately 3,600 µmol·kg⁻¹ dry muscle of acetyl groups is transferred from the small mitochondrial CoASH pool (<50 µmol·kg⁻¹ dry muscle) to the considerably larger cytosolic free carnitine pool [15–20 mmol·kg⁻¹ dry muscle] each minute during intense exercise [43].

The rapid CAT reaction during intense muscular contraction and, subsequently, the maintenance of a viable mitochondrial CoASH pool have a profound impact on skeletal muscle energy metabolism. In the first instance, maintaining PDH activation allows cytosolic pyruvate to be committed to an oxidative rather than anaerobic fate, therefore limiting lactate formation during exercise. This has been demonstrated experimentally where so-called mitochondrial inertia, i.e., the lag in mitochondrial ATP production at the onset of muscular contraction that is accompanied by phosphagen depletion and lactate production, has been shown to reside at the level of the PDH [44–48]. Indeed, maximally activating the PDH pharmacologically with dichloroacetate, an antagonist of the kinase that inhibits PDH activation, attenuates lactate formation and phosphagen degradation at the onset of muscle contraction [44]. Furthermore, Wall and colleagues have recently shown that elevating muscle carnitine content in humans increases PDH flux and acetylcarnitine formation during exercise at 80% of VO₂ max [49]. In addition, augmented PDH flux in the study of Wall et al. was accompanied by reduced lactate accumulation. Taken together, these findings underscore the role of carnitine and the CAT reaction in securing an oxidative fate for pyruvate and maintaining mitochondrial ATP production during high-intensity exercise by acting as an acetyl group buffer within skeletal muscle.

To briefly summarize this section on carnitine’s role in carbohydrate metabolism during intense exercise, it is clear that when the PDH nears its maximal activation status, it can produce acetyl-CoA at a rate that is far in excess of its condensation with oxaloacetate in the TCA cycle. In theory, this poses a significant problem with regards to mitochondrial bioenergetics, as excessive acetyl-CoA production, as in the production of acetyl-CoA at a rate above that of its consumption by citrate synthase, may acetylate the small pool of CoASH within the mitochondria. The metabolic consequences of this would be inhibition of enzymes within the mitochondria that consume CoAH, such as CPT-2, PDH, and α-ketoglutarate dehydrogenase. Put plainly, the generation of electrochemical energy from the TCA cycle and electron transport chain would cease, meaning that ATP production would be maintained by glycolysis and phosphagen depletion alone. Subsequently, without acetylcarnitine formation, intense muscular
contraction would only be sustainable for a matter of seconds. Therefore, free carnitine availability and the CAT reaction play a critical role in skeletal muscle bioenergetics during intense contraction.

An overview of carnitine’s role in maintaining a viable mitochondrial CoASH pool and committing pyruvate to an oxidative fate via the accumulation of acetylcarnitine during near maximal intensity exercise is depicted schematically in Figure 3.2.

**Carnitine as a regulator of skeletal muscle fuel selection**

A fundamental characteristic of skeletal muscle is its ability to oxidize both glucose and fatty acids, and to be able to switch between the two fuel sources depending on substrate availability, redox state, metabolic demand, and circulating hormone levels. In the early 1960s, a group of biochemists at the University of Cambridge in the UK proposed a model in the rat diaphragm muscle whereby fatty acid oxidation regulated glucose uptake and oxidative disposal, the premise for this being allosteric inhibition of the PDH enzyme complex by acetyl-CoA derived from the oxidation of fatty acids [41]. Further, an additional component of Randle and coworkers’ thesis was that acetyl-CoA condensation with oxaloacetate in the TCA cycle and a subsequent increase in citrate concentrations inhibited glycolysis at the level of phosphofructokinase [41].

*Figure 3.2* The role of carnitine as an acetyl group buffer.
Chapter three: Integration of skeletal muscle fuel metabolism

Although the regulatory mechanisms outlined in the Randle cycle still hold true today under many circumstances, contrary to Randle’s classical view, it has been shown that hyperglycemia and hyperinsulinemia can attenuate long-chain (CPT-1-dependent) fatty acid oxidation in vivo. Using a combination of stable and radioactive isotopes to trace long- and short-chain fatty acid metabolism, researchers were able to demonstrate that inhibition of CPT-1 (with either etomoxir or insulin) abolished long-chain fatty acid oxidation while having no measurable impact on medium-chain fatty acid oxidation in rodents and humans [50, 51], where oleic acid (CPT-1-dependent) oxidation was almost completely abolished while octanoic acid (CPT-1-independent) oxidation remained unchanged during a hyperglycemic hyperinsulinemic clamp [51]. The same group went on to demonstrate that impaired CPT-1-dependent long-chain fatty acid oxidation was associated with a threefold increase in skeletal muscle malonyl-CoA content during the hyperglycemic hyperinsulinemic clamp [52]. Malonyl-CoA is an intermediate in de novo lipogenesis in lipogenic tissues as well as being a potent CPT-1 inhibitor in the liver [53]. Considering that skeletal muscle is not a lipogenic tissue, this strongly suggests that malonyl-CoA, via inhibiting CPT-1 activity, inhibits skeletal muscle long-chain fatty acid oxidation when glucose availability is plentiful.

Taken together, the aforementioned studies demonstrate the reciprocal relationship between fat and carbohydrate oxidation in skeletal muscle. Interestingly, carnitine acts as a substrate for the two enzymes that are considered as the rate-limiting steps of fat (CPT-1) and carbohydrate (PDH) oxidation. Accordingly, carnitine occupies a unique position within skeletal muscle substrate metabolism, where free carnitine availability may limit fat or carbohydrate oxidation, dependent on several factors including ATP turnover rate, fuel availability, and circulating hormone levels.

Exercise provides an ideal setting to study the role that muscle carnitine availability plays in the integration of fuel selection as it provides a model where muscle ATP turnover can be increased by increasing exercise intensity. Further, it is well known that there is a relative and absolute shift from predominantly fat to carbohydrate oxidation on the continuum from rest to near maximal intensity exercise [54–56], which is largely mediated by increased glycolytic and PDH flux. Indeed, PDH activation and flux during intense exercise results in the rapid acetylation, and thus the transient depletion of the free carnitine pool [36–38, 42, 57]. Moreover, van Loon et al. demonstrated that during high-intensity submaximal exercise a 35% reduction in long-chain fatty acid oxidation was accompanied by a 65% decline in the muscle free carnitine pool, which was accounted for by an increase in muscle acetylcarnitine content [56]. These interesting findings offered a plausible mechanistic explanation as to why fat oxidation declines at high exercise intensities, where a temporary acetylation of muscle free carnitine becomes limiting to CPT-1
flux, thus impairing long-chain fatty acid transport into the mitochondrion and subsequent oxidation. Further to the study of van Loon and coworkers, others have also shown that PDH activity and a subsequent transient decrease in muscle free carnitine attenuates fat oxidation during moderate-intensity exercise in the glycogen-loaded state. Roepstorff and colleagues showed that elevating muscle carbohydrate availability (glycogen) prior to exercise at 65% of VO$_2$ max resulted in a 50% reduction in free carnitine content [54]. Similar to previously reported data from van Loon et al., this decline in muscle free carnitine content was accompanied by a concurrent accumulation of muscle acetylcarnitine and resulted in a 2.5-fold attenuation in long-chain fatty acid oxidation when compared to a non-glycogen-loaded control group [54]. These findings subsequently suggest that exercise intensity and carbohydrate availability may attenuate long-chain fatty acid oxidation via greater glycolytic and subsequently PDH flux, where the free carnitine pool becomes temporarily consumed by the CAT reaction.

The example of the relative and absolute shift from fat to carbohydrate oxidation during high-intensity exercise and the corresponding increase in acetylcarnitine and depletion of free carnitine stores provides the most compelling demonstration of carnitine's dual role in skeletal muscle fuel metabolism. One interpretation is that free carnitine simply acts as a substrate for CPT-1 or PDH, depending upon ATP turnover rates and glycolytic flux. So as the rate of glycolysis increases in response to increases in ATP demand, carnitine is consumed by the CAT reaction in order to maintain PDH flux and a preferable mitochondrial acetyl-CoA-to-CoASH ratio. In support of this, muscle carnitine loading in humans at rest or a moderate-intensity exercise (where PDH activation is not maximal) results in glycogen sparing [49, 58], suggesting that elevated muscle carnitine levels increase CPT-1 activity when energy demand and glycolytic flux are low. In contrast, though, Wall and colleagues have recently demonstrated that during submaximal high-intensity exercise where PDH activation was maximal, carnitine loading resulted in elevated PDH flux [49]. Here it would seem that a greater energetic demand, and thus greater carbohydrate oxidation, during intense exercise, results in free carnitine being consumed by the CAT reaction.

**Summary remarks**

Carnitine plays two distinct roles in skeletal muscle fuel metabolism, where it acts as a substrate for both CPT-1 and CAT. This dual role of carnitine in muscle fat and carbohydrate oxidation makes it an interesting compound, as altering the availability of carnitine within muscle potentially offers an opportunity to intervene in two distinct metabolic pathways. By way of example, elevating muscle carnitine levels in clinical populations...
characterized by lipid storage myopathies may increase CPT-1 activity and thus fatty acid oxidation. In the context of sports nutrition, elevating carnitine levels in skeletal muscle may increase fatty acid oxidation and thus spare glycogen at lower exercise intensities, or better maintain aerobic ATP production at near maximal intensity exercise. A caveat to use of carnitine supplementation is that even long-term dosing does not readily augment muscle carnitine content owing to the considerable concentration gradient between the plasma and muscle carnitine pools. In Chapter 4, skeletal muscle carnitine transport will be described and discussed in detail by Dr. Stephens. Thereafter, the impact of altering muscle carnitine levels on substrate metabolism will be addressed by Dr. Wall (Chapter 5) before the role of carnitine supplementation in specific clinical and athletic populations is discussed by a number of experts in the field of carnitine.

References


chapter four

Skeletal muscle carnitine transport

Francis Stephens

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Introduction

In line with the metabolic roles of carnitine highlighted in earlier chapters, the concentration of carnitine within a tissue tends to be dependent upon the oxidative capacity of the tissue. Skeletal muscle has high oxidative capacity to cope with the energy demands of physical activity, and therefore has a high carnitine concentration. Coupled with the fact that skeletal muscle comprises a large proportion of body mass, more than 95% of the total body carnitine pool resides within skeletal muscle. The enzyme responsible for the final step of carnitine biosynthesis, butyrobetaine hydroxylase, is not expressed in skeletal muscle, and so carnitine must be imported from its sites of endogenous synthesis and dietary absorption via the circulation (see Chapters 1 and 2). However, the circulating plasma carnitine concentration is approximately 100-fold less than that of skeletal muscle (50 vs. 5000 μM), and so carnitine must be actively transported into the tissue. This process is therefore crucial to whole body carnitine homeostasis and will form the basis of this chapter.
Characterization of skeletal muscle carnitine transport

Rebouche (1977) was the first to report that carnitine uptake into skeletal muscle against such a large concentration gradient is mediated by an active transport system that is Na\(^+\) dependent. Experiments performed in rat extensor digitorum longus muscle in vitro demonstrated a high-affinity saturable L-carnitine transport process, with an apparent Michaelis-Menten constant (\(K_m\)) of 60 μM and maximal rate (\(V_{\text{max}}\)) of 20 nmol h\(^{-1}\)g\(^{-1}\) tissue. Transport was inhibited by ouabain (a potent Na\(^+\)-K\(^+\) ATPase pump inhibitor), Na\(^+\) depletion, and L-carnitine analogues containing a quaternary ammonium group such as D-carnitine, butyrobetaine, and choline. The Na\(^+\)-dependent nature of muscle carnitine transport was also confirmed by studies performed using plasma membrane vesicles isolated from rat muscle homogenates (Berardi et al., 2000) and isolated rat myotubes (Georges et al., 2000), being increased with K\(^+\) preloading and demonstrating inhibition by ouabain and coenzyme A depletion (coenzyme A is essential for ATP production). \(K_m\) values for L-[\(^3\)H]carnitine uptake in these studies of 13.1 and 6.4 μM, respectively, and \(V_{\text{max}}\) values of 60 and 10 nmol h\(^{-1}\)g\(^{-1}\) muscle tissue, respectively, were comparable to those of isolated rat muscle by Rebouche (1977). A major conclusion from these studies was that there did not appear to be any Na\(^+\)-independent muscle carnitine transport, suggesting only a single carnitine transport mechanism in skeletal muscle. This is in contrast to other tissues such as the small intestine, where the ATB\(^{0,+}\) amino acid transporter also has L-carnitine activity, and the heart, where an antiport system is also active. For example, Sartorelli et al. (1982) demonstrated antiport systems in rat heart slices whereby intracellular butyrobetaine or acetylcarnitine was exchanged for extracellular carnitine. It has also been speculated that this latter process occurs in skeletal muscle to meet its demand for carnitine by synthesizing and exporting its precursor, or to remove toxic acyl groups and replenish carnitine that has been acetylated, particularly during intense exercise (see Chapters 1 and 2). However, Berardi et al. (2000) could not demonstrate L-carnitine uptake into skeletal muscle cells preloaded with butyrobetaine, and plasma acetylcarnitine does not increase measurably during exercise when millimolar concentrations of acetylcarnitine are produced intracellularly. Nevertheless, if an antiport system matched the turnover of intracellular carnitine, which within skeletal muscle is around 1 week, it may not be measurable over an acute experiment. Furthermore, in patients with systemic primary carnitine deficiency (SCD), an autosomal recessive disorder where there is a lack of carnitine transporter activity in tissues, muscle carnitine content can be restored via L-carnitine supplementation. Indeed, in some instances muscle carnitine content can be less than 1% of normal, and a
short period of L-carnitine supplementation can reverse the associated exercise intolerance and muscle pain and weakness (Treem et al., 1988; Tein et al., 1990). At first this may seem paradoxical if there is no muscle carnitine transporter activity, but this would suggest that in the absence of high-affinity carnitine uptake, intracellular carnitine can passively follow extracellular concentrations by diffusion down a concentration gradient (Stanley et al., 1987) or a low-affinity antiport system may exist. More research in this area is clearly required.

**Novel organic cation transporter 2 (OCTN2)**

Further insight into skeletal muscle transport characteristics was provided with molecular identification of the carnitine transporter by Tamai and colleagues in 1998. Tamai et al. (1998) cloned the novel organic cation transporter 2 (OCTN2) from a human kidney cDNA library and identified it as the physiologically important, high-affinity Na\(^+\)-dependent carnitine transporter in humans. This was based upon the observed tissue distribution of OCTN2, which was consistent with the reported distribution of carnitine transport activity, and the functional characteristics of OCTN2, which coincided with those reported for plasma membrane carnitine transport. For example, in agreement with the aforementioned studies, Tamai et al. (1998) demonstrated that in expression studies of OCTN2 in HEK293 cells, uptake of L-[\(^3\)H]carnitine was strongly enhanced in a Na\(^+\)-dependent manner (transport was abolished when Na\(^+\) was removed) with \(K_m\) and \(V_{max}\) values of 4.3 μM and 60 nmol·h\(^{-1}\)·g\(^{-1}\) protein, respectively. Expression of OCTN2 in HEK293 cells also demonstrated transport of structural L-carnitine analogues, such as D-carnitine, acetyl carnitine, butyrobetaine, and other acyl carnitines. L-Carnitine transport was inhibited in the presence of these compounds, with longer-chain acylcarnitines producing the greater inhibition (Ohashi et al., 1999). Interestingly, intracellular long-chain acylcarnitine accumulation would likely reflect detrimental excess mitochondrial fatty acid oxidation, and inhibiting carnitine uptake into the cell could perhaps provide a theoretical scenario to prevent further fat oxidation. Stoichiometric analysis demonstrated that L-carnitine transport was electrogenic, due to coupling with the Na\(^+\) gradient, and suggested that one Na\(^+\) was associated with the transport of one carnitine molecule (Hill coefficient of 0.93) (Tamai et al., 1998; Ohashi et al., 1999), and expression of mutated OCTN2 in HeLa cells resulted in complete loss of carnitine transport function (Seth et al., 1999). Northern and Western blot analyses have confirmed that OCTN2 is expressed in skeletal muscle tissues (Tamai et al., 1998; Wu et al., 1999; Furuichi et al., 2010), and immunohistochemical analysis of rat skeletal muscle cells has demonstrated that muscle contraction may translocate OCTN2 to the plasma membrane, increasing L-[\(^3\)H]carnitine uptake by 50% (Furuichi et
al., 2012). This latter finding is fascinating and suggests that muscle carnitine transport can be increased to meet cellular carnitine demand, particularly during intense physical exercise when its availability is limiting. Indeed, OCTN2 is a member of the solute carrier family (SLC22a5), 557 amino acids in length with 12 putative transmembrane spanning domains (Wu et al., 1999), which is similar to other translocating transporters such as the facilitative glucose transporter family member 4 (GLUT4). However, the concentration of total carnitine within human skeletal muscle does not increase during physical exercise, and there does not appear to be any carnitine efflux from the muscle, so it is unclear why carnitine transport would need to increase during exercise. On the other hand, it appears that OCTN2 content and L-[³H]carnitine flux within rat skeletal muscle are directly proportional to the percentage of type 1 fibers within that tissue (Furuichi et al., 2012). This would be consistent with the premise that carnitine content is greater in more oxidative tissues, although this is not the case in rat or human skeletal muscle where there is no difference in total carnitine content between type 1 and 2 fibers. Perhaps carnitine transport via OCTN2 is more important for compartmentalization of carnitine within the cell (see Chapters 1 and 2). Indeed, it has been demonstrated in a series of eloquent experiments that OCTN2 is also expressed on the basolateral surface of the vascular endothelium in the heart (Grube et al., 2006), presumably in order to concentrate carnitine in the extracellular space. If this is also the case for skeletal muscle, then this may explain why there is no difference in total carnitine measured in whole homogenates of muscles containing different fiber types. A large extracellular carnitine concentration could also explain the rapid efflux of carnitine from skeletal muscle homogenates observed under conditions such as hemodialysis. Again, further research is required.

Definitive confirmation of OCTN2 as the protein responsible for carnitine transport in vivo, and its physiological significance, was presented by Nezu and colleagues (1999), who were the first to demonstrate that patients with SCD have a loss of carnitine transporter function caused by mutations in the gene encoding OCTN2 (SLC22a5). Unfortunately, OCTN2 is also required for efficient renal reabsorption, and so patients with SCD have greatly increased carnitine excretion by the kidney, further limiting the availability of carnitine in the body and exacerbating the deficiency (Treem et al., 1988). Many cases of defective OCTN2 have been reported in patients with SCD, with several different missense mutations, although the same genetic defect does not always display the same phenotype, suggesting that environment (e.g., dietary or placental carnitine availability) also plays an important role (Wang et al., 2000).
Human skeletal muscle carnitine transport in vivo

The in vivo characteristics of human skeletal muscle carnitine transport are still not known, as to date there have been no studies of muscle carnitine transport in humans, just studies of net carnitine balance. However, comparisons to the carnitine transport studies in animal models can still be examined. For example, Soop et al. (1988) investigated carnitine exchange across the leg in healthy young men at rest and during 2 h of exercise at 50% of maximal oxygen uptake ($V_{O_{2\text{max}}}$) using an arteriovenous balance technique. As expected, there was no exchange of free or acylated carnitine at rest, but during exercise there was around a $2\mu\text{mol}\cdot\text{min}^{-1}$ net release of free carnitine and a $1\mu\text{mol}\cdot\text{min}^{-1}$ net uptake of acylcarnitine. The significance of this is unclear, as it was calculated that in line with the finding that muscle total carnitine content does not change measurably during exercise, the net loss of carnitine during exercise would equate to less than 2% of the muscle pool. This finding would suggest that OCNT2 translocation with exercise observed in rat skeletal muscle does not occur in humans to increase carnitine uptake during exercise, although it is important to note that this technique only measures balance and not transport. Furthermore, if we assume that the net uptake of acylcarnitine was all transported into the active muscle (reported around 20 kg) via OCTN2 (for which acylcarnitine is also a substrate), then over the 2 h of exercise muscle OCTN2 transport activity would have equated to $3\text{ nmol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ muscle tissue, which is considerably lower than that observed in the rodent studies described above. Inferences of human skeletal muscle carnitine transport in vivo can also be made from studies where muscle carnitine accumulation is measured following L-carnitine administration. For example, just 4 weeks of daily L-carnitine feeding increased rat skeletal muscle carnitine content by up to 50%, whereas neither feeding L-carnitine daily for up 3 months (Barnett et al., 1994; Vukovich et al., 1994; Wächter et al., 2002), nor intravenously infusing L-carnitine for up to 5 h (Brass et al., 1994; Stephens et al., 2006a) had an effect on muscle total carnitine content, or indeed net uptake of carnitine across the leg (Soop et al., 1988). Taken together, it is clear that although human skeletal muscle carnitine transport processes are similar between rodents and humans, the characteristics of transport may differ, which probably reflects lower carnitine concentration in rodent skeletal muscle (1 vs. 5 mM), and the dramatically different reliance on substrate utilization.

A primary reason for L-carnitine feeding failing to impact upon total muscle carnitine content, and therefore muscle energy metabolism, in humans is undoubtedly the poor bioavailability of orally administered L-carnitine (<20% for a 2- to 6-g dose). However, the finding that intravenous L-carnitine administration also has no impact on muscle carnitine content suggests that muscle carnitine transport is most likely the limiting
factor to muscle carnitine accumulation in healthy humans, and is the primary reason why it is difficult to study muscle carnitine uptake in vivo. Indeed, as stated above, the $K_m$ for carnitine of human OCTN2, in vitro, is 4.3 μM (Tamai et al., 1998), which would suggest that in the basal state skeletal muscle carnitine uptake is saturated, and it is unlikely that plasma carnitine availability per se will be rate limiting to muscle carnitine transport and storage (plasma carnitine concentration is ~40 μmol·L⁻¹). Thus, simply increasing plasma carnitine availability would not increase muscle carnitine transport. Moreover, even if we assume that the in vivo $K_m$ and $V_{max}$ for carnitine of human muscle carnitine transport are closer to those seen for isolated rat muscle of 60 μM and 20 nmol·h⁻¹·g⁻¹, respectively (Rebouche, 1977), then over a 5-h intravenous L-carnitine infusion where the circulating plasma carnitine concentration is maintained eightfold higher than the $K_m$ for carnitine transport (500 μM) (Figure 4.1) we could only expect

![Figure 4.1](image-url)  

*Figure 4.1* Plasma free carnitine concentration during intravenous L-carnitine infusion ($t = 1$ h) in the presence of a fasting (open circles) or a physiologically high serum insulin concentration (160 mIU·L⁻¹; closed circles). Values are means ± SEM of combined data from Stephens et al. (2006a, 2007a) ($n = 17$). ***, $p < .001$; **, $p < .01$, significantly less than fasting insulin visit.
to see a 100-μmol·kg⁻¹ (2%) increase in muscle carnitine content. For this reason, a great deal of research has been focused on ways of increasing skeletal muscle carnitine transport activity in order to measurably increase muscle carnitine content as a tool to study muscle carnitine metabolism (see Chapter 5).

**Insulin stimulates human skeletal muscle carnitine transport**

The 1:1 stoichiometry of Na⁺/carnitine co-transport into muscle and the fact that it is inhibited by ouabain would suggest that perhaps increasing sarcolemmal Na⁺/K⁺ ATPase activity, and therefore Na⁺ flux, might increase muscle OCTN2 carnitine transporter activity. Indeed, the finding that the kinetics of carnitine transport in isolated rat myotubes did not show any time-dependent binding activity (Georges et al., 2000), and that the in vitro $K_m$ and $V_{max}$ of OCTN2 for carnitine in rodents are lower than in isolated whole muscle tissue, would suggest that some cytosolic or membrane-associated activities are required for full carnitine uptake activity. With this in mind, it has been hypothesized that insulin could augment Na⁺-dependent skeletal muscle carnitine transport via OCTN2 (Stephens et al., 2006a), secondary to its action of increasing sarcolemmal Na⁺/K⁺ ATPase pump activity (Sweeney and Klip, 1998; Clausen, 2003). For example, an increase in Na⁺/K⁺ ATPase activity would lower the intracellular Na⁺ concentration, which would increase the electrochemical gradient for Na⁺, and therefore favor Na⁺/carnitine co-transport. In support of this hypothesis, the Na⁺-dependent uptake of other nutrients, such as some amino acids (Zorzano et al., 2000) and creatine (Steenge et al., 1998) by skeletal muscle, is augmented by insulin. It also cannot be ruled out that insulin could increase OCTN2 translocation to the plasma membrane in a similar way to that seen following contraction in rodent skeletal muscle. Thus, a series of experiments by Stephens and colleagues demonstrated that hypercarnitinemias (~500 μmol·L⁻¹) combined with hyperinsulinemias (~160 mU·L⁻¹) achieved via intravenous infusion of L-carnitine (1 μmol·kg⁻¹·min⁻¹) and insulin (105 mU·m⁻²·min⁻¹), respectively, reduced steady-state plasma carnitine concentration and resulted in an increase in skeletal muscle total carnitine content of around 15% (Stephens et al., 2006a, 2006b) compared to control (L-carnitine infusion during a fasting circulating insulin concentration, or saline infusion during hyperinsulinemia). This clearly suggested that insulin could increase skeletal muscle carnitine transport. Furthermore, as there was no difference in urinary carnitine excretion between high and low circulating insulin, the lower steady-state plasma carnitine concentration observed during hyperinsulinemia could be used to calculate carnitine uptake into tissues. For example, the intravenous L-carnitine infusion rate during hyperinsulinemia was 80 μmol·min⁻¹. The plasma free carnitine concentration was at a steady state throughout
this infusion period (470 μmol·L⁻¹) (Figure 4.1), and therefore the rate of L-carnitine infusion was equal to its rate of elimination, i.e., 80 μmol·min⁻¹. The rate of carnitine clearance from the plasma over the infusion period can therefore be calculated as 170 ml·min⁻¹ (the rate of elimination divided by the steady-state plasma concentration). The renal clearance calculated during hyperinsulinemia was approximately 100 ml·min⁻¹, equating to a 70-ml·min⁻¹ difference between plasma and renal carnitine clearance. Since the rate of tissue uptake equals the plasma concentration × plasma clearance, it can be calculated that plasma carnitine was transported into skeletal muscle at a rate of 33 μmol·min⁻¹ under insulin stimulation. This would equate to 60 nmol·h⁻¹·g⁻¹ muscle tissue (assuming 35 kg of muscle mass in the volunteers), which is threefold greater than the $V_{\text{max}}$ previously reported in rat tissue and certainly feasible given that insulin has been reported to double Na⁺/K⁺ ATPase activity (Sweeney and Klip, 1998; Clausen, 2003). We can also estimate from the above calculations that skeletal muscle carnitine content would have increased by around 10%, which was comparable to the 15% increase measured in muscle biopsy samples taken before and after L-carnitine infusion, particularly given that the vastus lateralis muscle biopsied has a higher carnitine content than other skeletal muscles in the body. Interestingly, by performing a similar experiment but with different insulin infusion rates (5, 30, 55, and 105 mU·m⁻²·min⁻¹) during hypercarnitinemia, it was demonstrated that plasma carnitine clearance was increased at a steady-state serum insulin concentration of around 90 mU·L⁻¹, but not 50 mU·L⁻¹, suggesting that a threshold concentration exists for the stimulatory effect of insulin on skeletal muscle carnitine accumulation (Stephens et al., 2007a).

A similar experiment conducted in vegetarian volunteers, who have reduced dietary carnitine intake (see Chapter 2), demonstrated that although basal skeletal muscle carnitine content was around 20% lower than in nonvegetarian controls, there was a delayed reduction in steady-state plasma carnitine concentration (5 vs. 3 h observed in nonvegetarians) (Figure 4.1) with insulin and no increase in muscle carnitine accumulation during 5 h of L-carnitine infusion (Stephens et al., 2011). This would seem paradoxical, as one might expect that tissue carnitine uptake capacity to be elevated in vegetarians in order to “scavenge” any available carnitine to maintain normal tissue carnitine content for physiological function. However, closer inspection revealed that the lack of uptake of L-carnitine into skeletal muscle of the vegetarian volunteers was most likely due to reduced muscle content of OCTN2, as evidenced by 33 and 37% lower OCTN2 mRNA expression and protein content, respectively, compared to nonvegetarian volunteers. This was in agreement with the aforementioned finding that the L-carnitine uptake capacity of rat skeletal muscle was directly proportional to the amount of muscle OCTN2 protein (Furuichi et al., 2010). Further insight can be provided by considering
the skeletal muscle OCTN2 mRNA expression response to L-carnitine infusion. For example, L-carnitine infusion in the presence of a fasting serum insulin concentration had no effect on OCTN2 mRNA expression in vegetarians and nonvegetarians, whereas it increased 2.3-fold during L-carnitine infusion in the presence of hyperinsulinemia in nonvegetarians where muscle total carnitine content increased by 15% (Stephens et al., 2006a, 2011). However, there was no change in OCTN2 mRNA expression in vegetarians under the same conditions where there was also no increase in muscle carnitine content. This also suggests that intracellular carnitine concentration may directly or indirectly regulate OCTN2 mRNA expression. Indeed, the peroxisome proliferator-activated receptor alpha (PPARα) is known to regulate OCTN2 gene expression in skeletal muscle (Ringseis et al., 2009), and an increase in skeletal muscle carnitine content has been shown to increase muscle long-chain acyl-CoA content and fatty acid flux (Stephens et al., 2006b, 2013), both of which are potent activators of PPARα (Hostetler et al., 2005).

The effects of insulin on skeletal muscle carnitine uptake have also been demonstrated with oral administration of L-carnitine in combination with large quantities of carbohydrate to stimulate insulin release in healthy human subjects. For example, a 3-g dose of L-carnitine followed by four 500-ml solutions containing 94 g of simple sugars at 1.5-h intervals resulted in 60 mg greater whole body retention of carnitine over 24 h compared to ingestion of L-carnitine alone (Stephens et al., 2007b). Furthermore, this effect was maintained throughout 2 weeks of daily L-carnitine (3 g) and carbohydrate feeding (2 × 500-ml solutions containing 94 g of simple sugars). Importantly, the physiologically high serum insulin concentration achieved in this experiment (~75 mU·L⁻¹) was in keeping with the predicted threshold at which an increase in muscle carnitine accumulation was thought to occur (i.e., between 50 and 90 mU·L⁻¹). Assuming that the greater whole body retention of L-carnitine following daily L-carnitine and carbohydrate feeding resided within skeletal muscle, it can be calculated that L-carnitine feeding in conjunction with carbohydrate ingestion would have increased skeletal muscle carnitine concentration by a further 0.3% (whole body muscle store is 20 g) compared with L-carnitine ingestion alone. Consequently, if maintaining a daily L-carnitine feeding regime with carbohydrate has an additive effect on muscle carnitine content, L-carnitine feeding for 30 days could increase muscle carnitine content by an additional 10%. Indeed, 12 weeks of twice daily L-carnitine (1.36 g) and carbohydrate (80 g) feeding (spaced 4 h apart for the peak in plasma carnitine concentration after the first drink to coincide with the peak in circulating insulin concentration after the second drink) increased muscle carnitine content by 20% (Stephens et al., 2013), which was further increased after 24 weeks (Wall et al., 2011), compared to carbohydrate feeding alone. Furthermore, there was also an increase in
muscle OCTN2 mRNA expression after 12 weeks, which appeared to be mediated via PPARα, as other PPARα-responsive genes were also upregulated (Stephens et al., 2013). More research into why and how intracellular carnitine regulates the transcription of its own transporter is required.

Summary
Carnitine transport into skeletal muscle appears dependent upon the amount of the high-affinity carnitine transporter OCTN2 in muscle. OCTN2 has a relatively low \( K_m \) compared to the plasma concentration such that simply increasing circulating carnitine is unlikely to further increase transport. Furthermore, OCTN2 has a relatively low \( V_{max} \) compared to the intramuscular carnitine concentration such that the rate of carnitine uptake into muscle, and its turnover, is low. To date, this appears to be the only carnitine transport mechanism into skeletal muscle unless the intramuscular concentration becomes depleted to such a degree that passive diffusion can occur, such as with L-carnitine supplementation in individuals with SCD. Nevertheless, it appears that under insulin, and possibly contraction, stimulated conditions the rate of carnitine uptake into muscle can be increased, which can result in an increase in the muscle carnitine pool if plasma carnitine concentration is elevated for a prolonged period of time. The mechanism behind this is likely an increase in \( \text{Na}^+ / \text{K}^+ \) ATPase pump activity increasing intracellular \( \text{Na}^+ / \text{carnitine} \) co-transport, but it could also be due to an increase in OCTN2 translocation to the plasma membrane or even an increase in OCTN2 transcription and translation. Ingesting carnitine every day for at least 12 weeks and ensuring that the peak plasma carnitine concentration coincides with a high circulating insulin concentration is, to date, the most practical way to measurably increase skeletal muscle carnitine content.

References


The metabolic impact of manipulating the skeletal muscle carnitine pool

Benjamin T. Wall

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The discovery that the skeletal muscle carnitine pool can be manipulated in animals and, more recently, humans has allowed great strides to be made in understanding the role of carnitine in muscle fuel metabolism. Moreover, the profound metabolic effects of modulating muscle carnitine availability have piqued the interest of those interested in the role that carnitine may play in human nutrition, both in various compromised clinical states and in the realm of sports nutrition. This chapter will build upon previous chapters, explaining the role that carnitine plays in the integration of fat and carbohydrate metabolism, and address the current understanding of how altering the muscle carnitine availability affects muscle fuel metabolism. Recent findings assessing both carnitine loading and depletion in human and animal models of health and disease will be considered. As such, the chapter aims to lay the mechanistic foundation for subsequent discussions on the (potential) role for carnitine in human nutrition.
Animal models of carnitine depletion

The juvenile visceral steatotic mouse lacks any activity of the organic carnitine cation transporter 2 (OCTN2) protein (the primary protein transporter permitting sodium-linked tissue carnitine uptake) and is consequently carnitine deficient [1]. As such, this phenomenon offers an effective model to observe the impact of carnitine deficiency on muscle fuel metabolism. These rodents exhibit skeletal muscle glycogen depletion and liver steatosis, both of which are readily reversed with carnitine supplementation [1]. These data suggest that carnitine deficiency per se leads to a greater reliance on carbohydrate-derived energy stores, presumably due to an inhibition of fat oxidation. However, with any genetically irregular species, it is important to consider that profound changes to global metabolism may occur, which cannot be entirely attributed to the availability of a single molecule. Thus, to further evaluate the specific role of carnitine in fuel metabolism, it is necessary to assess direct interventions designed to specifically deplete carnitine in animals.

Sodium valproate is a commonly used antiepileptic drug that leads to side effects such as hyperammonemia, encephalopathy, and hepatotoxicity [2, 3]. These side effects are thought to occur due to sodium valproate leading to muscle carnitine deficiency. Sodium valproate is a branched chain carboxylic acid with a chemical structure similar to that of fatty acids. As such, it is thought that the sodium valproate acts as a high-affinity substrate for carnitine-palmitoyltransferase 1 (CPT1) and the subsequent formation of valproyl-carnitine results in excessive renal carnitine loss in the form of valproyl-carnitine [3–5]. These observations have led to the use of sodium valproate as an experimental tool to investigate muscle fuel metabolism in animals deficient in muscle carnitine [6]. For instance, it was demonstrated that sodium valproate administration in mice led to impairments in in vivo long-chain fatty acid oxidation, reduced mitochondrial function (isolated preparations), and substantial accumulation of lipid in the liver [6]. Furthermore, these results were exacerbated when repeated in transgenic mice already exhibiting partial carnitine deficiency [6].

More recently, mildronate 3-(2,2,2-trimethylhydrazinium propionate) has been used as a more direct pharmacological strategy to induce carnitine depletion in animals [7]. Mildronate is a structural analogue of the carnitine precursor butyrobetaine and acts as a competitive inhibitor of butyrobetaine hydroxylase activity (the enzyme responsible for the final stage of endogenous carnitine biosynthesis), and consequently blocks hepatic carnitine biosynthesis [8, 9]. Furthermore, mildronate also acts as a competitive substrate for OCTN2 [10]. This leads to impaired renal carnitine reabsorption [8], increased carnitine urinary excretion [11–13], and reduced skeletal muscle carnitine uptake in vitro [14]. In keeping with these observations, it has been demonstrated on numerous occasions that
mildronate administration in animals results in significant depletion of the carnitine stores in plasma, heart, liver, and skeletal muscle tissue [8, 11, 12, 15–23].

Clinically, mildronate was directed as a drug to treat angina. It was shown that mildronate administration alleviates cardiac dysfunction in rat cardiac muscle, although the mechanism was not determined [19, 24]. It was suggested that mildronate may have reduced fat oxidation (evidenced by reduced accumulation of acyl-carnitines) and the oxygen cost of energy metabolism, thereby alleviating angina-related symptoms brought on by ischemia/hypoxia [19, 24, 25]. It was subsequently shown in rats, treated orally with mildronate, that near-complete carnitine depletion in the liver was accompanied by a halving of fatty acid oxidation in the isolated, perfused organs [26]. Importantly, mildronate-induced skeletal muscle and liver carnitine depletion has been shown to result in a halving of whole body in vivo fat oxidation (assessed via intraperitoneal injection of $^{14}$C palmitate and the subsequent collection of expired $^{14}$CO$_2$) [8, 12, 15]. Carnitine depletion also appears to result in cellular adaptations to the lipid metabolic machinery. For instance, while mildronate-induced carnitine depletion reduces in vivo lipid oxidation [15], it actually up-regulates in vitro CPT1 activity, and also increases state 3 (ADP-dependent) mitochondrial respiration when ample substrate is provided [15]. These data suggest that the mitochondria quickly adapt to the reduced availability of cellular carnitine, in an (inadequate) effort to maximize long-chain fatty acid oxidation.

Fat and carbohydrate stores share a reciprocal relationship with respect to their utilization, and regulate one another via mechanisms described in the Randle cycle [27, 28]. Thus, a decline in fat oxidation would be expected to be paralleled by an increase in carbohydrate utilization. In line with this, mildronate administration has been shown to decrease blood glucose concentration, increase tissue glucose uptake, and up-regulate the expression of genes related to glucose metabolism [20, 29]. In agreement, oral mildronate treatment resulted in decreased fatty acid oxidation accompanied by increased lipid stores and reduced glycogen content in isolated liver tissue [15], and reduced in vivo postabsorptive and postprandial blood glucose levels [18, 21]. Nevertheless, direct measurements of carbohydrate oxidation, or metabolic intermediates determined in skeletal muscle following carnitine depletion, are, at present, lacking, and represent a natural area for future research.

Regardless of the method, low muscle carnitine levels result in impaired fat oxidation. Since skeletal muscle quantitatively makes up the primary tissue contributing to whole body fat oxidation, muscle carnitine depletion is likely of most relevance when considering whole body energy metabolism. However, it is important to acknowledge that while mildronate-mediated carnitine depletion has been documented in the mixed-fiber gastrocnemius [30] and quadriceps femoris [12, 23], the majority of
data from similar studies has been obtained from nonmuscle tissues. As such, there is much to be learned, especially from a mechanistic point of view, of the role of carnitine depletion in skeletal muscle energy metabolism. Carnitine depletion seems to result in a compensatory acceleration of carbohydrate utilization, a point worthy of additional comment. The inhibition of CPT1 as a method to lower blood glucose levels (by increasing glucose oxidation) has been tentatively suggested as a potential therapeutic strategy for insulin resistance and type 2 diabetes [31–33]. However, while mildronate-induced lowering of blood glucose appears to have similar beneficial effects, the longer-term impact of potently inhibiting fatty acid oxidation is largely unknown. Likely effects include liver steatosis [12, 15, 23, 34], hyperlipidemia [15], and the accumulation of intramuscular lipids, which all would be expected to exacerbate insulin resistance [35]. Indeed, mildronate is often used by researchers to develop a murine model of steatosis. Moreover, it would appear that many of these effects worsen over time [18, 21]. As such, inhibition of CPT1 or depletion of carnitine as a strategy to improve insulin resistance should be treated with some caution and requires further investigation. Taken together, the available data from animals depleted of tissue carnitine highlight the obligatory part that carnitine plays in fat oxidation, and its central role in the regulation of muscle fuel selection. However, the precise mechanisms by which carnitine depletion inhibits fat oxidation in vivo have not yet been fully explored.

Carnitine deficiency in humans

As outlined in the previous section, depleting carnitine in vivo leads to numerous undesirable metabolic consequences. As such, specifically depleting the carnitine pool in humans is not (yet) a viable experimental strategy. Nevertheless, some data are available in humans who are, at least partly, carnitine deficient either due to natural phenomena (i.e., systemic carnitine deficiency), as an indirect result of clinically warranted pharmacological interventions (e.g., sodium valproate), or as a consequence of certain clinical conditions/treatments (e.g., end-stage renal failure/dialysis). Such conditions necessarily present with a multitude of changes in metabolism, not necessarily linked to carnitine deficiency. Specific conditions associated with disturbed carnitine metabolism, and the potential for carnitine to be used as a nutritional therapy, will be addressed in later chapters. However, clues to the impact of carnitine depletion on fuel metabolism and energy homeostasis can be found from some studies investigating natural or pharmacologically induced carnitine deficiency in humans.

Systemic (or primary) carnitine deficiency results from an autosomal recessive mutation in the gene coding the OCTN2 protein [36]. Patients exhibiting this disorder are incapable of reabsorbing carnitine in the
kidney due to this process being entirely dependent on OCTN2-mediated active transport. In addition, these patients obtain negligible quantities of carnitine from the diet since carnitine absorption from the gut is also dependent upon the action of OCTN2. Accordingly, systemic carnitine deficiency results in a skeletal muscle carnitine content approximately 1% of that found in healthy controls [37, 38]. Resultant effects include lipid accumulation within both cardiac and skeletal muscle, severe muscle weakness, and exercise intolerance, effects that are most profound in more oxidative muscle (more reliant on lipid oxidation for energy production than more glycolytic muscle) [37, 38]. Moreover, similar to the juvenile visceral steatotic mouse model described earlier, these effects are, to a large extent, reversed by carnitine supplementation [37, 38], again suggesting that carnitine depletion directly inhibiting fat oxidation is the primary cause of the metabolic problems suffered by these patients.

Sodium valproate administration in humans has been shown to reduce plasma and tissue concentrations of carnitine [39, 40]. Reported side effects include microvesicular steatosis, elevated amino transaminase levels, and hyperammonemia [4]. These effects are reflective not only of a reduced ability to oxidize lipid stores, but also how this impairs the cellular energy status. Again, the importance of carnitine per se can be seen by studies that show that carnitine supplementation in parallel with sodium valproate administration reduces ammonia production and hepatotoxicity compared with sodium valproate administration alone [41–43]. Studies employing alternative, more direct approaches to deplete tissue carnitine stores in humans (e.g., mildronate administration) are currently restricted to a single study [13], and therefore metabolic insight is not yet available.

Thus, while controlled studies specifically depleting carnitine in human muscle tissue are currently lacking, data do exist from carnitine-deficient humans that highlight the obligatory requirement for carnitine in fat oxidation, and therefore the central role that carnitine occupies in muscle fuel metabolism. Moreover, the relative success that carnitine supplementation has in reversing or attenuating such complications will be further explored in later chapters.

**Carnitine loading in the skeletal muscle of animals**

It was demonstrated in the early 1990s that high-dose, bolus, intravenous injections of carnitine were able to transiently (for <48 h) and modestly (12%) elevate the muscle carnitine store in rats [44]. Around this time, most studies involving carnitine administration to animals were more concerned with establishing the pharmacokinetics, whole body metabolism, and dietary fate of exogenous carnitine [44, 45]. In contrast, less attention was paid to the metabolic consequences of increasing tissue carnitine content. However, two studies in particular, performed in the early 1990s, laid
the foundation for our understanding of the metabolic impact of muscle carnitine loading.

In 1991, it was demonstrated that the exposure of dog latississimus dorsi (mixed-fiber type) muscle to high-level carnitine (infusions) during an in situ model of muscle contraction resulted in greater isometric force development and a delay in the onset of fatigue [46]. However, since no change in muscle carnitine content was observed following the relatively brief infusion period, the mechanism behind this improvement in function was not clear. The authors speculated that it could be extramyo-cellular, particularly since the in situ model allowed for an intact vasculature [46]. Aiming to extend on these data, seminal work from Brass and colleagues was published 2 years later [47]. Here, the authors chose to take an in vitro model of muscle contraction using isolated rat muscle strips. Again, it was found that exposure of the muscle tissue to high levels of carnitine resulted in a delay in fatigue development during contraction. However, this improvement in muscle function was dependent on a preincubation of the muscle strips with carnitine, which resulted in an approximate fivefold increase in the muscle store of carnitine prior to contraction. The authors therefore suggested that the extremely high carnitine gradient in this model allowed for muscle carnitine accumulation via diffusion, and that this effect was necessary for improved muscle function. Furthermore, while the metabolic mechanism was still unknown, it was also observed that carnitine resulted in glycogen sparing, an increase in long-chain acyl-CoA concentration, and only exerted its beneficial effect in muscle comprising predominantly type I fibers (soleus) and not in faster muscle extensor digitorum longus (EDL). Taken together with the observations of Dubelaar et al. [46] that carnitine only benefited muscle function in the absence of insulin (which would drive carbohydrate-derived energy metabolism), it was speculated that carnitine likely exerted its effect via an interaction with mitochondria and, specifically, by driving CPT1-mediated fatty acid oxidation.

While these studies provided the framework for which future human investigations would be based, many scientists prematurely assumed administering carnitine to humans would also result in muscle carnitine loading. As such, much scientific effort was directed at carnitine feeding as a means to positively influence muscle metabolism in healthy and disease states. However, as described in Chapter 4, muscle carnitine stores in humans are remarkably resistant to both oral supplementation and intravenous infusions. Thus, it was not until the last decade, when the kinetics of intramuscular carnitine transport were better understood, that data were generated looking at the metabolic impact of muscle carnitine loading in humans.
Carnitine loading in human skeletal muscle

The first demonstration of skeletal muscle carnitine loading in humans occurred in 2006 [48]. Thereafter, the same researchers were able to show the impact on fuel metabolism in resting skeletal muscle [49]. In this experiment, it was shown that a ~15% increase in muscle carnitine content (achieved via the intravenous infusion of carnitine during a hyperinsulinemic euglycemic clamp) led to an attenuation of the insulin-induced increase in lactate accumulation and pyruvate dehydrogenase complex activity (PDCa) in healthy men. Lactate accumulates due to an acceleration of glycolytic flux; i.e., carbohydrate-derived pyruvate formation exceeds the capacity for decarboxylation to acetyl-CoA by the PDC, and thus pyruvate becomes reduced to lactate. The PDC is an enzyme complex viewed as the rate-limiting step in carbohydrate oxidation, where pyruvate becomes committed to an oxidative fate as acetyl-CoA within the tricarboxylic acid (TCA) cycle [50, 51]. Accordingly, in this seminal human experiment, the authors concluded that glycolytic flux and glucose oxidation were attenuated in the carnitine-loaded state. Moreover, it was also observed that carnitine loading led to an increased overnight storage of muscle glycogen and an accumulation of fatty acyl-CoAs. Thus, mechanistically, it was speculated that the inhibition of carbohydrate oxidation had occurred specifically at the level of PDC due to increased acetyl-CoA delivery from fat oxidation [28], and this led to glucose being directed away from oxidation and toward storage [49]. As such, the findings from this study also have clear implications for the improvement of insulin sensitivity in metabolically compromised populations.

While this early study clearly demonstrated that carnitine loading attenuated carbohydrate utilization, an increase in fat oxidation was not measured. Moreover, from a health or performance perspective, augmenting fat oxidation is usually most desirable during exercise. As such, further research went on to assess the effect of carnitine loading on skeletal muscle fuel selection during aerobic exercise [52, 53]. It was reported that a ~20% elevation of muscle carnitine content (via long-term oral supplementation protocols) increased whole body fat oxidation rates during low- to moderate-intensity aerobic exercise (50% VO$_{2\text{max}}$) [53]. Additionally, and in line with previous resting studies [49], a reduced muscle PDCa was also observed in combination with a halving of muscle glycogen utilization during exercise [52]. Pathway-focused, quantitative, real-time polymerase chain reaction (PCR)-based low-density array analyses also provided additional insight into the metabolic pathways altered at the gene expression level [53]. Specifically, compared to a control group, functional units of genes concerned with insulin signaling, peroxisome proliferator-activated receptor (PPAR) signaling, and fatty acid metabolism were the most enriched pathways in the carnitine-loaded condition [53].
The enriched insulin signaling gene cluster supports previous data [49] suggesting that carnitine loading may have a beneficial effect on insulin sensitivity, even in healthy volunteers. The up-regulation of PPAR signaling and fatty acid metabolism pathways offers a detailed explanation of how fatty acid flux is accelerated with carnitine loading. Indeed, with carnitine loading, 5 of the 6 most enriched transcripts of those analyzed (187 in total) concerned key proteins in the fat metabolic pathway, namely, adipose triglyceride lipase (ATGL) (intramyocellular lipid hydrolysis), CPT1 (fatty acid entry into mitochondria), acetyl-coenzyme acetyltransferase 1 (ACAT1) (terminal \( \beta \)-oxidation), pyruvate dehydrogenase kinase 2 (PDK2) (inhibition of carbohydrate oxidation), and Forkhead box O3 (FOXO3) (transcriptional regulator of PDK expression). PPAR\( \alpha \) is also the main transcriptional regulator of CPT1 in skeletal muscle, and this gene was twofold greater expressed with carnitine loading than the control. Taken together, the study provided strong in vivo evidence not only that carnitine loading augmented fat oxidation during exercise, but that this likely occurred through an increased flux through the CPT1 reaction and a consequent, adaptive up-regulation of key genes in the fat metabolic pathway (i.e., lipolysis, intramitochondrial transport, \( \beta \)-oxidation).

Increasing fat oxidation during low-intensity exercise has important implications for health promotion. However, of additional interest from an exercise metabolism and exercise performance point of view, is increasing the rate of fat oxidation during higher-intensity, submaximal exercise [54]. It has frequently been hypothesized that increasing muscle carnitine content prior to exercise above 75% maximal oxygen uptake (VO\(_{2\text{max}}\)) may be an effective way of increasing fat oxidation, sparing muscle glycogen stores, thus improving performance capacity [54–57]. However, data thus far have proved unable to test this hypothesis [52]. On the contrary, recent data looking at carnitine loading prior to high-intensity, submaximal exercise have actually been able to better define the importance of carnitine to maintain carbohydrate oxidation [52]. As discussed in previous chapters, when PDC flux is increased such that acetyl-CoA formation outweighs its incorporation into the TCA cycle (e.g., during high-intensity, submaximal exercise), carnitine also acts as a buffer for the excess acetyl groups produced. This is achieved by the formation of acetylcarnitine via the action of carnitine acetyltransferase (CAT), thus ensuring a viable cellular pool of free coenzyme A [58–60]. It was reported that during exercise at 80% VO\(_{2\text{max}}\), volunteers in the carnitine-loaded state displayed a profound increase in PDC activation and flux [52]. This resulted in a reduction in muscle lactate accumulation, and thus a better maintenance of cellular energy status. Thus, carnitine loading in the face of high rates of glycolytic and PDC flux did not increase mitochondrial fatty acid translocation and subsequent oxidation. Rather, the importance of these data was the demonstration that carnitine loading augmented the matching of PDC
and TCA cycle fluxes, and consequently improved skeletal muscle oxidative (as opposed to nonoxidative) adenosine triphosphate (ATP) production. The fact that these specific metabolic adaptations also translated into greater exercise performance points to the relevance of carnitine loading in sports nutrition or in clinical conditions associated with impaired oxygen delivery to the muscle (e.g., peripheral vascular disease). It remains to be seen whether a threshold exists for the maximum possible increase in muscle carnitine, such as that observed with creatine supplementation [61]. Thus, whether further increases in muscle carnitine than previously reported would saturate carnitine’s involvement in the CAT reaction and therefore increase its availability to CPT1 and augment fat oxidation during high-intensity, submaximal exercise is currently unknown.

The studies mentioned above [48, 52, 53] have described the impact of carnitine loading on acute muscle fuel metabolism at rest and during differing intensities of exercise. However, a fascinating additional consideration is the effect that elevating the muscle carnitine pool has in the longer term on energy expenditure and body composition. Carnitine loading has been shown to increase total energy expenditure in humans during low-intensity exercise [53]. This was attributed to the oxidation of fat being less efficient than carbohydrate for ATP production, and therefore a carnitine-induced increase in fat oxidation requiring more substrate for the same energy generation [53]. It was not defined whether such an increase in energy expenditure would also occur at rest. However, it was reported that 6 months of excess carbohydrate intake, resulting in ~2 kg gain in body fat in a control group, was entirely prevented in the carnitine-loaded condition [53]. As such, carnitine-mediated increases in fat oxidation and total energy expenditure during exercise (and possibly at rest) result in better maintenance of body composition in the face of excess caloric intake. Naturally, these findings are of great relevance when considering the development of nutritional strategies to treat type 2 diabetes and obesity, especially given the possibility that carnitine loading also directly improves insulin sensitivity [49, 53].

Conclusions

Over the past half century, the basic biochemistry concerning the metabolic reactions for which carnitine is required within skeletal muscle has been well described. More recently, animal and human models employing various approaches to manipulate muscle carnitine levels have provided great insight into the importance of carnitine for muscle fuel metabolism. The majority of these studies have highlighted the crucial role that carnitine plays in CPT1-mediated fatty acid transport into the mitochondria for subsequent β-oxidation. Specifically, the depletion of carnitine greatly impairs fat oxidation rates with an apparent compensatory increase in
carbohydrate utilization. This results in the accumulation of excess lipids within muscle and other organs, and is also associated with muscle weakness and fatigue. On the other hand, carnitine loading leads to an enhanced fatty acid oxidative capacity, more efficient use of carbohydrate stores during intense contraction, and improved exercise performance. Moreover, emerging evidence suggests that in the longer term, carnitine loading increases energy expenditure during exercise and helps to maintain body composition, as well as profoundly modulating gene networks associated with insulin signaling. The remainder of this book will consider these mechanistic data in the context of human nutrition, and specifically address several clinical or healthy conditions associated with metabolic disturbance where carnitine metabolism/nutrition is of relevance.

References


section three

Applications of carnitine in human nutrition
Carnitine metabolism and nutrition in dialysis patients

Eric P. Brass

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l-Carnitine (l-3-hydroxy-4-N-trimethylammonium butyrate, hereafter referred to as carnitine) is a key cofactor in cellular biochemistry and whole body energy metabolism [1–3]. The kidney plays an important role in normal carnitine homeostasis. Thus, it is not surprising that renal disease is associated with changes in whole body carnitine homeostasis. Further, renal disease is associated with important systemic metabolic changes that may also influence carnitine metabolism. The profound changes in carnitine homeostasis in patients with renal disease have led to hypotheses that carnitine supplementation may have therapeutic value in patients with renal dysfunction. Understanding whole body carnitine homeostasis, the function of the kidney in carnitine metabolism and metabolic derangements associated with renal failure are required to interpret the clinical data available on the therapeutic use of carnitine in these patients.
Carnitine homeostasis and the kidney—normal physiology

Carnitine homeostasis is complex. Carnitine is derived from both dietary sources and endogenous biosynthesis. There is no catabolism of carnitine in mammalian cells, but it is reversibly converted to acylcarnitines in reactions catalyzed by a family of carnitine acyltransferases [4, 5] (Figure 6.1). The pool of acylcarnitines will consist of a spectrum of specific compounds defined by the fatty acyl moiety attached to carnitine (for example, acetylcarnitine, propionylcarnitine, etc.). Thus, the carnitine content in a biological compartment is described by the concentrations of carnitine, each individual acylcarnitine (when not otherwise specified, “acylcarnitine concentration” refers to the sum of all individual acylcarnitine concentrations), total carnitine (the sum of carnitine and all acylcarnitines), and the distribution of the total carnitine between carnitine and acylcarnitines. The distribution of the carnitine pool between carnitine and

\[
\text{Acyl-CoA + Carnitine} \rightleftharpoons \text{Coenzyme A + Acylcarnitine}
\]

Figure 6.1 The reversible transfer of fatty acyl groups from coenzyme A to carnitine. The reaction shown is catalyzed by a family of carnitine acyltransferases [5] and is readily reversible conserving the energy of acyl group activation. In most tissues the reaction appears to operate near equilibrium as the distribution of acyl groups in the coenzyme A and carnitine pools is similar. This concordant redistribution of the carnitine and coenzyme A (CoA) pools is seen in human skeletal muscle during high-intensity exercise (acetylcarnitine increases from 28% to 70%, calculated as percent of the sum of carnitine + acetylcarnitine, while acetyl-CoA increases from 25% to 51%, calculated as percent of the sum of coenzyme A + acetylcarnitine, in the transition from rest to exercise at 90% of VO\textsubscript{2max}). (Data adapted from Constantin-Teodosiu et al., \textit{Acta Physiol Scand} 1991;143:367–72.)
acylcarnitines is important, as the acyltransferase reaction appears to operate near equilibrium in most mammalian tissues. As a result, the distribution of a tissue’s carnitine pool reflects the status of the metabolically critical coenzyme A (CoA) pool. Thus, when a metabolic transition occurs leading to increased concentration of an acyl-CoA, the corresponding acylcarnitine will also accumulate at the expense of carnitine (Figure 6.1). This reaction is particularly important under conditions of metabolic dysfunction where generation of the acylcarnitine can lower the concentration of a potentially toxic acyl-CoA and make CoA available for other reactions [5–7].

Carnitine or acylcarnitines do not readily cross plasma membranes. Transport systems facilitate carnitine and acylcarnitine movements across cellular membranes and differ in expression from one tissue to another. The novel organic cation transporter-2 (OCTN2) is particularly important for the uptake of carnitine by cells [8, 9]. Thus, each tissue compartment may have different carnitine and acylcarnitine concentrations and will equilibrate with the plasma compartment at different rates, dependent on the transporters expressed and the metabolic state of the tissue. Over 90% of the body’s total carnitine is in muscle [10, 11]. The muscle compartment equilibrates only slowly with plasma, and thus acute changes in plasma are poorly reflected in the muscle carnitine pool [12–15]. In contrast, the liver carnitine-acylcarnitine distribution is readily reflected in plasma [16, 17].

The kidney plays a central role in integrating homeostasis (Figure 6.2). In humans, the kidney contains significant activity of the last enzyme in carnitine biosynthesis, butyrobetaine dioxygenase (also known as butyrobetaine hydroxylase), although the enzyme is present in other tissues [18]. Carnitine and acylcarnitines in the blood are filtered through the glomerulus and enter the tubular lumen. Under physiologic conditions over 95% of the filtered carnitine is reabsorbed [19, 20], primarily through OCTN2. The importance of OCTN2 in human carnitine homeostasis is evidenced by the severe clinical phenotypes associated with OCTN2 mutations [21]. Acylcarnitines are also reabsorbed, although to a lesser degree than carnitine [20]. However, interpretation of urinary acylcarnitines concentrations is complex, as acylcarnitines generated by metabolism in renal tubular cells can also appear in the urine [22]. Further, reabsorption of acylcarnitines is variable based on acyl chain length, as shorter-chain-length acylcarnitines may be more effectively reabsorbed than those with longer chain lengths [23]. Tubular reabsorption of carnitine is also saturable, and at concentrations above approximately 60–90 μM carnitine excretion markedly increases [24, 25]. At very high plasma carnitine concentrations urinary carnitine clearance approaches the glomerular filtration rate [15, 19].
Changes in carnitine homeostasis in renal failure

An understanding of carnitine homeostasis in patients with renal disease requires consideration of total carnitine concentrations in tissue compartments of interest as well as the distribution of the carnitine pool between carnitine and specific acylcarnitines. Serum plasma carnitine concentrations are elevated in patients with renal insufficiency in whom dialysis therapy had not been initiated [26–28], consistent with decreased renal clearance [28]. Additionally, these patients have increased plasma acylcarnitine concentrations [27, 29]. The increase in plasma acylcarnitines in these patients likely reflects a combination of deceased renal clearance [29] and altered whole body metabolism [27]. Tissue carnitine content was not measured in these studies, but muscle carnitine content is normal in patients in whom dialysis therapy has recently been initiated [30, 31], suggesting that muscle carnitine content is also normal in patients with chronic renal insufficiency not on dialysis.
Carnitine homeostasis is more complex in patients on hemodialysis. Changes in diet may reduce oral intake of carnitine and biosynthetic precursors as carnitine is preferentially found in meat and dairy products [3]. Loss of renal parenchyma may theoretically decrease carnitine biosynthetic capacity as butyrobetaine dioxygenase activity is lost. However, no comprehensive studies of carnitine biosynthesis have been done in patients on hemodialysis. In a cross-sectional study, Yllmaz Selcuk and colleagues reported that hemodialysis patients on supplementation with methionine and lysine had higher serum carnitine concentrations than those not on supplements, suggesting lower biosynthesis due to precursor deficiency [32]. However, plasma concentrations of the carnitine biosynthetic precursor 6-N-trimethyllysine [33] have been reported to be normal in patients on hemodialysis. As availability of trimethyllysine is thought to be rate limiting in carnitine biosynthesis [34, 35], this would suggest no impairment in patients with end-stage renal disease. Direct assessment of butyrobetaine concentrations, a carnitine biosynthetic intermediate distal to trimethyllysine and the substrate for renal butyrobetaine dioxygenase, can be viewed as contradicting this conclusion as butyrobetaine concentrations have been reported to be decreased in patients on hemodialysis [36]. Butyrobetaine is removed by dialysis, complicating the interpretation of this observation [36]. Additionally, the comparison of butyrobetaine concentrations with nonhemodialysis subjects was made using literature values and methodological differences might have contributed to the reported differences. Finally, as butyrobetaine availability may not be rate limiting for carnitine biosynthesis, the implications of these observations for understanding carnitine homeostasis are unclear.

Patients on hemodialysis will have little or no urinary carnitine excretion but will lose carnitine and acylcarnitines during each dialysis session. During a dialysis session plasma carnitine concentrations drop dramatically as carnitine is removed in the dialysate. Plasma carnitine and acylcarnitine concentrations at the end of a 2-hour hemodialysis may be only 20–30% of those at the initiation of dialysis [37]. However, plasma carnitine and acylcarnitines increase between dialysis sessions such that by 48 hours plasma carnitine concentrations are no different than predialysis concentrations [37,38]. This increase between dialysis sessions reflects reequilibration of the plasma compartment with the large intracellular carnitine stores in tissues, particularly muscle. The movement of carnitine between tissues and plasma is slow, and these kinetics explain the uncompensated decrease during dialysis and the slow restoration between sessions [38].

In the absence of transplantation, hemodialysis is a lifelong commitment for patients with end-stage renal disease. Thus, the cumulative net impact of dialysis losses may be important. Careful mass balance studies have tried to address this issue and suggest that in patients on thrice
weekly hemodialysis, weekly total carnitine losses in dialysate are similar to or less than weekly urinary carnitine losses in healthy subjects [39], but small net losses could not be definitively excluded.

Thus, the integrated effects of changes in diet, biosynthesis, and carnitine/acylcarnitines elimination are difficult to predict. Early studies reported that plasma carnitine concentrations were low in patients on hemodialysis, suggesting carnitine deficiency [40,41]. However, more comprehensive analyses demonstrated that plasma acylcarnitines concentrations were elevated in hemodialysis and plasma total carnitine concentrations were normal or elevated [27,42].

Acylcarnitine accumulation in plasma may reflect both abnormal acylcarnitine production and decreased acylcarnitine clearance. While many acylcarnitines are efficiently cleared during hemodialysis, acylcarnitines with acyl moieties larger than eight carbons are not well extracted, contributing to their accumulation [43,44]. Additionally, to the degree cellular metabolism is impaired in end-stage renal disease patients, acyl-CoA metabolic intermediates may accumulate and be removed as the corresponding acylcarnitines [27]. These concepts are supported by the observation that unusual and longer-chain acylcarnitines are found in the plasma of hemodialysis patients [30,45]. Indeed, the longer a patient has been on dialysis, the higher the concentrations of these nonacetyl acylcarnitines in plasma [30]. Redistribution of the carnitine pool toward a specific group of acylcarnitines due to a metabolic impairment has been best characterized in inherited metabolic disorders [46,47]. Importantly, it has been demonstrated that in these conditions carnitine requirements for removing the metabolic intermediates may be supra-physiologic, leading to these conditions being characterized as “carnitine insufficiency” and providing an additional rationale for carnitine therapy in these conditions [47,48].

Carnitine homeostasis is highly compartmentalized, and the plasma may be a poor reflection of the muscle carnitine pool. Changes in total carnitine or the distribution between carnitine and acylcarnitines may occur in one compartment and not be reflected in another. For example, acute increases in plasma carnitine concentration have no measurable acute impact on muscle carnitine content in healthy subjects [15]. Similarly, the marked redistribution of the muscle carnitine pool toward acylcarnitines that occurs in healthy subjects during intense exercise is not reflected in the plasma carnitine pool [49]. Studies have now shown that in patients on hemodialysis skeletal muscle total carnitine content is inversely correlated with the length of time the patient has been on dialysis [30,31]. However, the redistribution of the carnitine pool toward acylcarnitines observed in plasma of hemodialysis patients is not seen in muscle [31,50]. An inverse relationship between muscle carnitine content and muscle function in hemodialysis patients has been suggested [31]. However, in
patients with carnitine deficiency due to transporter mutations, low muscle carnitine concentrations achieved with supplementation have been reported to restore normal strength [51], suggesting a more complex relationship between carnitine content and function.

Hepatic carnitine and acylcarnitines concentrations have not been measured in patients on hemodialysis. The liver compartment equilibrates with the plasma compartment more quickly than skeletal muscle [13]. Studies in animal models suggest that changes in hepatic metabolism leading to a shift in the hepatic carnitine–acylcarnitine distribution are rapidly reflected in the plasma carnitine pool [17]. Thus, it is possible that the hepatic metabolic state plays an important role in the accumulation of acylcarnitines in the plasma of patients on hemodialysis.

Patients on peritoneal dialysis have been less extensively studied. Carnitine is removed in the peritoneal dialysate [39]. Patients on peritoneal dialysis show the same redistribution of the plasma carnitine pool toward acylcarnitines as observed in hemodialysis patients [52, 53].

Taken together these data support possible decreased carnitine intake or decreased carnitine biosynthesis, combined with dialysis losses of carnitine and acylcarnitines, as leading to small net carnitine losses that result in potentially important total-body carnitine deficits over time. Additionally, metabolic changes likely contribute to a redistribution of the carnitine pool toward acylcarnitines. As discussed below, the clinical implications of the changes in tissue carnitine content are difficult to establish.

**Carnitine as a biomarker in patients on hemodialysis**

End-stage renal disease is associated with diverse abnormalities in patient well-being and function. Given the central role of carnitine in cellular function, many investigators have sought to identify relationships between a patient’s carnitine status and aspects of their clinical status. This approach has yielded a number of reports describing correlative relationships as illustrated in Table 6.1. As with any correlation, causality cannot be inferred from these relationships even when biologic plausibility can be established. Thus, the carnitine measurements can be considered biomarkers of the clinical parameter and may suggest mechanistic or therapeutic hypotheses.

**Effect of carnitine supplementation on carnitine homeostasis in patients on hemodialysis**

Carnitine supplementation has been best studied when given intravenously at the end of a hemodialysis session. Administration prior to the
dialysis would be expected to result in large losses of the administered carnitine in the dialysate. In healthy subjects after intravenous carnitine administration the majority of the dose is rapidly excreted in the urine as the tubular reabsorption threshold is exceeded [15, 19]. In contrast, in patients on hemodialysis given carnitine postdialysis the plasma carnitine concentration falls more slowly, likely reflecting distribution into tissue compartments [54]. During subsequent dialysis sessions the plasma carnitine and acylcarnitines concentrations fall as observed for patients not on carnitine supplementation [38, 54], and a substantial amount of the administered carnitine is recovered in the dialysate [36]. This cycle is repeated with each dialysis session and postdialysis carnitine dosing. Due to the slow turnover of the deep tissue compartments steady-state carnitine concentrations are only reached after 8 weeks [54] or longer [55]. Increases in muscle carnitine content ranging from 20% to over 100% in hemodialysis patients receiving long-term (6 weeks or greater) intravenous carnitine therapy have been reported [56–59]. At the high plasma

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Carnitine parameter</th>
<th>Example of study demonstrating association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulatory functional status</td>
<td>Inverse correlation with ratio of plasma acylcarnitines concentration to plasma carnitine concentration</td>
<td>Riley et al. [75]—using Karnofsky activity score (n = 31)</td>
</tr>
<tr>
<td>Peak exercise capacity</td>
<td>Direct correlation with muscle total carnitine content</td>
<td>Hiatt et al. [31]—muscle total carnitine content inversely correlated with duration of dialysis (n = 8)</td>
</tr>
<tr>
<td>Erythropoietin dose requirements</td>
<td>Inverse correlation with serum total carnitine concentration</td>
<td>Matsumura et al. [76]—also correlated with erythrocyte fragility (n = 26)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Directly correlated with increased contribution of longer-chain acylcarnitines in plasma carnitine pool</td>
<td>Murphy et al. [45]—longer-chain acylcarnitines also inversely correlated with physical performance (n = 58)</td>
</tr>
<tr>
<td>Handgrip strength</td>
<td>Directly correlated with plasma carnitine concentration</td>
<td>Constantin-Teodosiu et al. [77]—relationship only statistically significant in female patients (n = 51)</td>
</tr>
</tbody>
</table>

**Note:** No effort has been made here to comprehensively review all clinical studies, but examples are provided for the clinical parameters listed.
Carnitine concentrations achieved after intravenous dosing plasma membrane transporters other than OCTN2 may contribute to tissue uptake. Additionally, exchange transports may allow for carnitine uptake into muscle in exchange for butyrobetaine or acylcarnitines [14,60]. The latter may be important in facilitating the removal of accumulating metabolic intermediates as acylcarnitines under these conditions.

As would be anticipated given the above discussion, carnitine therapy impacts the plasma acylcarnitines pool in patients on hemodialysis. The plasma acylcarnitine concentration increases dramatically with intravenous carnitine therapy as the high acylcarnitines–carnitine ratio characteristic of end-stage renal disease is maintained despite the large increase in total carnitine [55]. The composition of the acylcarnitines pool is also influenced during carnitine treatment. In one study, prior to carnitine treatment acetylcarnitine contributed only approximately 20% of the plasma acylcarnitines compared with >70% in healthy subjects [61] (Table 6.3). Over a course of 24 weeks of treatment the contribution of acetylcarnitine increased to 40% of the total acylcarnitines [61] (Table 6.3). Carnitine treatment is also associated with large increases in plasma butyryl-, isobutyryl- isovaleryl-, octanoyl-, and decanoyl-carnitine concentrations [36,61]. These data confirm that carnitine treatment in hemodialysis patients results in increased delivery of carnitine to metabolically active compartments and enhances the removal of acyl groups generated during cellular metabolism. The large contribution of nonacetyl acylcarnitines supports the hypothesis that intermediates of incomplete oxidative metabolism accumulate in the cells of these patients, which can be effectively removed with carnitine therapy [27,61]. The increased contribution of acetylcarnitine to the total acylcarnitines over time with treatment suggests a normalization of the metabolic environment during treatment. The clinical significance of these changes is unknown.

**Therapeutic use of carnitine in patients on hemodialysis**

The hypothesis that carnitine supplementation would have therapeutic value in patients with end-stage renal disease is attractive given the dramatic changes in carnitine metabolism in patients on hemodialysis and the correlations between these changes and clinically important symptoms and signs (Table 6.1). Further, two plausible mechanistic hypotheses suggest that carnitine supplementation may be clinically efficacious in dialysis patients. First, carnitine may be required to replenish tissue stores depleted during years of end-stage kidney disease. Second, pharmacological amounts of carnitine may be required to optimize the removal of accumulating intermediates associated with disordered metabolism in
end-stage renal disease. Despite large numbers of clinical trials that have been well reviewed [62–66], there remains no consensus as to the therapeutic benefit of carnitine therapy in hemodialysis patients or in which patients it should be used. Most studies have used intravenous carnitine at doses of approximately 20 mg/kg body weight given after each dialysis session. Examples of endpoints reported improved by carnitine therapy are highlighted in Table 6.2.

The literature on therapeutic use of carnitine is challenging to read. Conducting trials in patients on hemodialysis is difficult given the complexities of their medical conditions and the relatively small number of patients (in the United States approximately 1,800 patients per million population [67]). There are many small studies showing benefit of carnitine therapy, raising the risk of publication bias, as small negative studies are less likely to be published. Research methodologies have often not been robust, as some studies lack control groups or identify benefits in subgroups of patients. The few large, well-conducted trials have often not confirmed benefits.

A review of studies related to exercise in patients on hemodialysis offers an example of the apparently contradictory results observed in clinical trials. As noted above, carnitine is important for skeletal muscle function, muscle carnitine content is decreased over time in patients on hemodialysis, and muscle carnitine content is correlated with exercise capacity in these patients [31, 50, 68]. One of the first large randomized studies of carnitine therapy in hemodialysis patients demonstrated in a subgroup of subjects that maximal exercise capacity increased with carnitine therapy as assessed as maximal oxygen consumption [69]. Yet, a subsequent larger study designed specifically to assess exercise capacity with carnitine therapy in hemodialysis patients failed to confirm the earlier results [55]. However, these two studies may not be as irreconcilable as their top-line results appear. The studies were done 20 years apart and background care of hemodialysis patients had changed dramatically, including the use of erythropoietin. Additionally, the more recent study found group differences in favor of carnitine using secondary statistical approaches, suggesting that a small benefit of carnitine could not be excluded [55].

The importance of carefully reviewing study details is also illustrated in clinical trials of carnitine to improve cardiac left ventricular function in patients on hemodialysis. In a double-blinded, randomized trial Fagher et al. [70] reported no effect of carnitine therapy on left ventricular ejection fraction. However, subsequent, unblinded, single-arm trials have reported that carnitine therapy improves left ventricular ejection fraction [71, 72]. The different results may reflect more than open versus blinded study designs. Fagher et al. [70] studied a cohort in which left ventricular function was relatively well preserved, while the single-arm studies studied only patients with markedly reduced left ventricular function.
<table>
<thead>
<tr>
<th>Study endpoint</th>
<th>Example of study showing benefit</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intradialytic symptoms and cramps</td>
<td>Bellinghieri et al. [78]—no control group</td>
<td>Meta-analysis does not confirm benefit and identifies weaknesses in studies [79]</td>
</tr>
<tr>
<td>Serum triglyceride concentrations</td>
<td>Guarnieri et al. [80]—approximately 30% reduction reported</td>
<td>Meta-analysis does not confirm [62], unclear clinical significance</td>
</tr>
<tr>
<td>Refractory anemia</td>
<td>Matsumoto et al. [81]—increase hematocrit in erythropoietin refractory patients given oral carnitine</td>
<td>Supported by meta-analysis [62], but patient selection criteria unclear and no definitive confirmatory study [82]</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Brass et al. [55]—12 or 24 weeks treatment improved scores on fatigue domain on Kidney Disease Questionnaire</td>
<td>Fatigue was a secondary endpoint in trial</td>
</tr>
<tr>
<td>Hospital utilization</td>
<td>Kazmi et al. [83]—11–15% reduction in hospitalization risk</td>
<td>Review of database—use of carnitine not on randomized trial basis</td>
</tr>
<tr>
<td>Muscle trophic effect</td>
<td>Giovenali et al. [58]—7% increase in type I and type IIa fiber diameters; no control group</td>
<td>No change in muscle strength in placebo-controlled trial [56]</td>
</tr>
<tr>
<td>Improved left ventricular ejection fraction</td>
<td>van Es et al. [71]—improved ejection function in patients with impaired cardiac function</td>
<td>No effect in randomized trial, but trial included patients with relatively preserved cardiac function [70]</td>
</tr>
<tr>
<td>Maximal exercise capacity</td>
<td>Ahmad et al. [84]—improved VO$_{2\text{max}}$ endpoint assessed in only subset of patients</td>
<td>Not confirmed in larger randomized trial [55]</td>
</tr>
<tr>
<td>Quality of life</td>
<td>Steiber et al. [85]—improvement on role-physical and physical component scores of SF-36</td>
<td>No effect on physical symptom score or overall score of Kidney Disease Questionnaire in large clinical trial [55]</td>
</tr>
</tbody>
</table>

*Note:* No effort has been made here to comprehensively review all clinical studies, but examples are provided for the endpoints listed.
While the single-arm design precludes a definitive causative association between carnitine therapy and the improved cardiac function, the results suggest that the earlier randomized study should not be considered the final word on a potential beneficial effect of carnitine on cardiac function.

In the United States carnitine is approved by the Food and Drug Administration for use in patients on hemodialysis to treat the biochemical derangements but without a clinical endpoint as part of the indication. Indeed, the reports of clinical benefits have been questioned [73]. A National Kidney Foundation Consensus Conference included erythropoietin-refractory anemia, intradialytic hypotension, heart failure, muscle weakness, and fatigue as potentially responsive to carnitine therapy in hemodialysis patients [74]. One way to interpret the breadth of data is that carnitine therapy is unlikely to be universally beneficial in hemodialysis patients and should not be considered as routine therapy. In contrast, in individual patients manifesting findings that have been reported to be carnitine responsive and who are refractory to other interventions, a trial of carnitine supplementation may be reasonable.

### Conclusions

Carnitine is associated with changes in carnitine homeostasis including an accumulation of acylcarnitines in plasma and progressive decreases in muscle total carnitine content in patients on long-term hemodialysis. Changes in carnitine metabolism can be correlated with several clinical sequelae of hemodialysis. However, it is not clear whether changes in carnitine metabolism are the cause of the clinical symptoms or a biomarker for the underlying pathophysiology. In particular, the accumulation of nonacetyl acylcarnitines may reflect disordered intermediary metabolism and incomplete substrate oxidation in these patients. Clinical trials of carnitine supplementation have not

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**Table 6.3** Change in plasma acylcarnitines in patients on hemodialysis treated with intravenous carnitine for 24 weeks

<table>
<thead>
<tr>
<th>Acylcarnitine</th>
<th>Baseline</th>
<th>12 weeks treatment</th>
<th>24 weeks treatment</th>
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<tbody>
<tr>
<td>Total acylcarnitines</td>
<td>15.9</td>
<td>80.3</td>
<td>109.3</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>3.44 (21.6%)</td>
<td>36.3 (45.2%)</td>
<td>46.4 (42.4%)</td>
</tr>
<tr>
<td>Isobutyrylcarnitine</td>
<td>0.39 (2.4%)</td>
<td>1.69 (2.1%)</td>
<td>1.44 (1.3%)</td>
</tr>
<tr>
<td>Isovalerylcarnitine</td>
<td>0.01 (&lt;0.1%)</td>
<td>0.59 (0.7%)</td>
<td>0.40 (0.4%)</td>
</tr>
</tbody>
</table>


*Note:* Patients (n = 10) received 20 mg/kg carnitine intravenously at the end of each dialysis treatment. Plasma samples were obtained prior to the start of the dialysis session. Concentrations are expressed as μmol/L with the percentage of total acylcarnitines contributed by the specific acylcarnitines shown in parentheses.
established unambiguous benefit in the general hemodialysis population. However, the underlying science and an overall assessment of the clinical trials suggest that carnitine therapy should be considered in individual patients in whom symptoms or signs of anemia, left ventricular dysfunction, intradialytic hypotension, or cramps, fatigue, or weakness occur and who are refractory to standard interventions. Disappointingly, available data are insufficient to definitively state the likelihood of success of carnitine therapy in these patients despite 30 years of research.

Disclosures

The author has been a consultant to Sigma-Tau Pharmaceuticals, which was a developer and marketer of carnitine for use in renal disease.

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chapter seven

Carnitine and insulin resistance

Shawna Wicks, Robert Noland, and Randall Mynatt

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Introduction

Among the more pressing health concerns of modern life is the explosive rise in rates of obesity, diabetes, and the metabolic syndrome. There is a
strong need for detailed mechanistic investigation that will pave the way for development of new preventive and treatment strategies. These must be both cost-effective to administer and free of potentially deleterious side effects with long-term treatment. Carnitine supplementation therapy has shown promising results in a limited number of human and animal trials, but several questions remain to be answered both practically and mechanistically. We first describe the current rationale for involvement of carnitine in diabetes and metabolic syndrome. Next, we briefly summarize existing animal and human studies of carnitine supplementation. We discuss in more detail mechanistic insights that have been gained both from carnitine supplementation and from genetic or pharmacological manipulation of the carnitine shuttle. Finally, we consider whether there is evidence for carnitine insufficiency in obesity/metabolic syndrome or diabetes that justifies the therapeutic use of supplementary carnitine. Looking forward to the future, we must identify patient characteristics associated with beneficial therapeutic outcomes. Key areas of research to accomplish this goal are highlighted.

Mechanisms of carnitine involvement in insulin resistance

Prime features of insulin resistance are reduced insulin-stimulated skeletal muscle glucose uptake, enhanced hepatic gluconeogenesis, and hepatic steatosis. Skeletal muscle accounts for approximately 80–90% of glucose uptake from the bloodstream (DeFronzo et al. 1985), and decades of research have focused on determining the limiting steps in this process. Excessive and dysregulated hepatic gluconeogenesis also plays a large role in fasting hyperglycemia once the disease progresses to a severe state (Boden et al. 2001; Basu et al. 2004). There are currently two popular theories on how lipid metabolism imbalance leads to derangements in insulin sensitivity; carnitine lies at the nexus of both (Mynatt 2009). The lipotoxicity theory suggests that decreased fat oxidative capacity results in ectopic lipid accumulation. Toxic by-products, such as diacylglycerols (DAGs) and ceramides, impair insulin signaling and GLUT-4 translocation to the membrane (Samuel et al. 2010; Samuel and Shulman 2012). Carnitine plays an essential role in facilitating fatty acid transport into the mitochondria via carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme in fatty acid oxidation (FAO). Thus, carnitine supplementation may improve insulin sensitivity by increasing the rate of FAO. The second theory suggests that oversupply of fatty acids to the mitochondria leads to accumulation of incompletely oxidized fat and impairment of glucose oxidation (Muoio and Neufer 2012). Here, too, carnitine may play a critical role. The enzyme carnitine acetyltransferase (CrAT) allows
efflux of excess acyl groups from the mitochondrial matrix in the form of short-chain acyl- and acetylcarnitines. Both theories are briefly summarized below, but the reader is directed to several excellent recent reviews for a more comprehensive treatment (Samuel et al. 2010; Fisher-Wellman and Neufer 2012; Muoio and Neufer 2012; Samuel and Shulman 2012). We focus instead on theoretical considerations and experimental evidence pertaining specifically to carnitine supplementation’s effects on insulin sensitivity, with discussion of how these results relate to the lipotoxicity and mitochondrial overload theories.

**Lipotoxicity**

Lipotoxicity occurs as a result of excess ectopic lipid accumulation (sometimes due to a deficiency in FAO) and has often been reported to contribute to the development of insulin resistance. However, although strong correlations between intramyocellular (IMCL) and hepatic lipid accumulation and insulin resistance drove much of the initial research (Pan et al. 1997; Krssak et al. 1999; Perseghin et al. 1999; Goodpaster and Kelley 2002; Erion and Shulman 2010), the theory is considerably more nuanced. For instance, endurance athletes are known to have both high IMCL and high insulin sensitivity, which is referred to as the athlete’s paradox (Krssak et al. 2000; Goodpaster et al. 2001). These seemingly contradictory findings led to additional studies. Findings from these studies indicate that it does not appear to be the accumulation of lipid, specifically triacylglyceride (TAG), per se, but rather elevations in specific lipid species that impair insulin signaling.

**DAGs and ceramides**

Much of the most convincing mechanistic work centers upon the ability of DAGs to activate novel protein kinase C (PKC), which then increases serine phosphorylation of IRS-1, blocking tyrosine phosphorylation and activation (De Fea and Roth 1997; Schmitz-Peiffer et al. 1997; Griffin et al. 1999; Ravichandran et al. 2001; Aguirre et al. 2002; Itani et al. 2002; Yu et al. 2002; Kim et al. 2004; Morino et al. 2008; Samuel et al. 2010). The other major lipid metabolite with purported negative effects on insulin signaling is ceramide, acting through multiple pathways downstream of IRS-1 (Schmitz-Peiffer et al. 1999; Stratford et al. 2004; Holland et al. 2007; Holland and Summers 2008). Ceramide activates a phosphatase that dephosphorylates Akt/PKB, blocking insulin-mediated GLUT-4 translocation (Long and Pekala 1996; Teruel et al. 2001; Chavez et al. 2003). Ceramide’s effects may also be intimately related to inflammation, and specific to saturated fatty acids (Shi et al. 2006; Ussher et al. 2010; Holland et al. 2011). While there have been many studies showing DAG accumulation, PKC
activation, and impaired insulin signaling, other studies have produced conflicting results. For instance, ATGL KO mice have pronounced ectopic lipid and increased DAG content in muscles, yet exhibit improved insulin signaling in skeletal muscle (Kienesberger et al. 2009). Similarly, DGAT overexpression increases DAG content, but improves muscle insulin sensitivity (Timmers et al. 2011). Knockdown of CGI-58, the ATGL activator, increases both hepatic DAGs and ceramides, while at the same time improving glucose tolerance and insulin sensitivity (Brown et al. 2010). These results suggest that there is a great deal more to learn with regard to lipid trafficking and localization within the cell that may be directly relevant to insulin signaling (Samuel and Shulman 2012).

**Impaired FAO**

The complex results obtained with disruption of lipid trafficking and storage highlight the regulatory role of lipid droplets in energy balance, and suggest that increased ectopic lipid research must consider changes to both energy demand and supply. The supply side of the equation has been well studied. Studies in humans utilizing magnetic resonance spectroscopy (MRS), indirect calorimetry, oxidative enzyme activities in biopsy specimens, fatty acid flux across the leg, and FAO in muscle strips appeared to support a theory of fat supply exceeding oxidative capability, showing decreased FAO, citric acid cycle flux, and adenosine triphosphate (ATP) synthesis, correlated with decreased mitochondrial density (Simoneau et al. 1995, 1999; Simoneau and Kelley 1997; Kim et al. 2000; Kelley et al. 2002; Hulver et al. 2003; Petersen et al. 2003, 2004; Morino et al. 2005; Befroy et al. 2007). Long-term high-fat feeding in rodents also impairs mitochondrial oxidative capacity and alters mitochondrial number and morphology, but this is a consequence, not cause, of insulin resistance (Bonnard et al. 2008). Indeed, recent rodent studies suggest that early stages of insulin resistance and obesity are characterized by enhanced, rather than diminished, flux through β-oxidation (Koves et al. 2005; Noland et al. 2007; Turner et al. 2007; Hancock et al. 2008). These results led to renewed interest in the concept of substrate competition and the role of energy balance, not just supply.

**Mitochondrial overload theory**

The idea of substrate competition in respiration is longstanding, but it is the classic experiments of the 1960s that led to the Randle hypothesis of fatty acid inhibition of glucose oxidation (Randle et al. 1963). In this model, it is proposed that increased FAO leads to accumulation of acetyl-CoA, NADH, and ATP that inhibit pyruvate dehydrogenase (PDH), blocking oxidation of glycolytic products. High levels of acetyl- and short-chain acyl-CoAs may also sequester CoA-SH, a vital cofactor in a multitude
of reactions (Ramsay and Zammit 2004; Zammit et al. 2009). Further, increased inhibition of phosphofructokinase-1 by citrate would decrease glycolysis (Denton and Randle 1966), resulting in hexokinase inhibition by glucose-6-phosphate. This theory lost popularity after Shulman and others proposed that muscle glucose disposal was limited by uptake, not phosphorylation of glucose (Shulman 2000; Muoio and Neufer 2012). This interpretation was strengthened by correlations between ectopic fat accumulation, decreased FAO, and insulin resistance, as described above. But, conflicting results have pointed out the necessity of analyzing impaired FAO and ectopic lipid accumulation with greater attention to regulation of mitochondrial oxidation by energy demand.

**Energy balance**

In recent years, there has been a resurgence of interest in the relevance of fatty acid-induced inhibition of glucose utilization due to both theoretical concerns and experimental data. It is a prime tenet of mitochondrial bioenergetics that the rate of respiration is controlled by the demand for ATP and the cost of maintaining the mitochondrial membrane potential (Brand 1990a, 1990b). Thus, rates of FAO “at rest” do not reflect the maximum oxidative capability of the mitochondria, and maximal FAO rates in ex vivo systems may not reflect in vivo energy demand or substrate interactions (Muoio and Neufer 2012). Experimentally, the initial response to a high-fat diet in rodents is upregulation of enzymes in the FAO system (Turner et al. 2007; Hancock et al. 2008). Subsequent development of decreased mitochondrial density may not suggest a decrement in the ability to oxidize sufficient supplied fuel, but rather a reduction of basal metabolic rate and a decreased need to oxidize fuel (Muoio and Neufer 2012). Paradoxically, transgenic mice with decreased oxidative capacity as a result of disruptions to a variety of mitochondrial enzymes or regulators often have increased insulin sensitivity (reviewed in Muoio 2010). These observations put the focus squarely on imbalances in mitochondrial oxidation and led to the mitochondrial overload theory. Briefly summarized, it postulates that:

1. In the initial stages of overnutrition, there is a high degree of incomplete FAO, since supply outstrips ATP demand (Hancock et al. 2008; Kraegen et al. 2008). This leads to accumulation of acylcarnitines and depletion of tricarboxylic acid (TCA) intermediates. This heightens the reducing potential in the mitochondria and also diminishes glucose oxidation by feedback inhibition of PDH (Muoio and Koves 2007; Power et al. 2007; Koves et al. 2008; Mynatt 2009; Noland et al. 2009).
2. Metabolic inflexibility (impairments in the ability to switch to fat oxidation during fasting and to glucose oxidation upon refeeding) results from overfueling of the mitochondria, persists in isolated
mitochondria, and is therefore at least partially independent of insulin signaling (Koves et al. 2008; Noland et al. 2009). Metabolic inflexibility is a major aspect of metabolic syndrome and insulin resistance (Kelley and Mandarino 2000; Thyfault et al. 2006).

3. The focus is not upon maximum oxidative capability as measured ex vivo, or upon resting oxidative function measured in vivo, but upon mitochondrial load (i.e., accumulation of acetyl-CoA and feedback inhibition of PDH). This puts the emphasis on the degree to which supply exceeds demand, and helps to explain otherwise counterintuitive studies where insulin sensitivity can be preserved or improved despite oxidative impairment or lipid accumulation.

It is worth noting that several of these studies use acylcarnitine profiling, either instead of or in addition to radiolabeled substrates, to provide a snapshot of intermediary metabolism. Acylcarnitine profiling generates a picture of flux through intermediary metabolism, and enables imbalances between complete and incomplete oxidation to be detected by accumulation of specific acylcarnitines (Boyle et al. 2011). As discussed by Muoio et al., a difficulty of the isotopic tracers used in many lipotoxicity studies is the possibility of label dilution due to the high endogenous lipid stores (Thyfault et al. 2010; Muoio and Neufer 2012). Acylcarnitine and acyl-CoA profiles provide a more complete picture of the in vivo situation at a given time.

Mitochondrial load and glucose uptake

1. Feedback inhibition. Recent studies using countertransport techniques, rather than nuclear magnetic resonance (NMR) and stable isotopes, suggest that control of glucose disposal may be split between glucose uptake via GLUT-4, glucose delivery to the cell, and glucose phosphorylation by hexokinase (Wasserman 2009). Once insulin signaling is disrupted, control resides at the level of GLUT-4 translocation. In earlier stages of insulin resistance, prior to profound disruption of the insulin signaling cascade, insulin is still able to induce GLUT-4 translocation, but glucose disposal is rate-limited by its phosphorylation and subsequent metabolism (Wasserman 2009). It is at this stage that feedback inhibition from PDH to hexokinase, as a result of mitochondrial overload, can impair glucose disposal.

2. Links to insulin signaling. To date, there is no direct, proven link between impairments to the insulin signaling cascade and mitochondrial overload. However, increased superoxide/hydrogen peroxide production by mitochondria respiring on fatty acids could alter the redox balance of the cell and change the activity of insulin signaling regulators with critical thiol residues, such as PTP1B (reviewed in Fisher-Wellman and Neufer 2012). Transcription factors linked
to insulin signaling, such as c-jun amino-terminal kinases (JNKs), KkB kinase catalytic subunit b (IKK-B), NF-kappaB (NF-kB), and protein kinase C (PKC), are known to be activated by oxidant stress (Chakraborti and Chakraborti 1998; Bloch-Damti and Bashan 2005). An elegant study from the Neufer laboratory linked excess H$_2$O$_2$ production to insulin resistance in rodents and humans (Anderson et al. 2009). Furthermore, the transcription of TXNIP is increased in diabetic rodent and human muscle, an event controlled by accumulation of glycolytic intermediates and hexokinase activity (Hui et al. 2008; Stoltzman et al. 2008; Chutkow et al. 2010; Sloan and Ayer 2010). TXNIP is able to decrease glucose uptake, but the mechanism remains to be defined (Parikh et al. 2007). Further research in these areas will help to determine if mitochondrial substrate load exerts effects on glucose disposal via parallel, independent mechanisms or by interaction with the canonical insulin signaling pathway.

Both the lipotoxicity theory and the mitochondrial lipid overload theory predict that carnitine supplementation has the potential to alleviate insulin resistance/metabolic syndrome/T2D, but by different mechanisms. Impairments to FAO, leading to accumulation of DAGs and ceramides, could be corrected by carnitine-mediated increases to FAO. Conversely, mitochondrial lipid overload could be minimized by carnitine-mediated efflux of excess acylcarnitines and preservation of free CoA. In the next section, we discuss several studies of carnitine supplementation or genetic alterations that attempt to define the mechanism of the therapeutic effect.

**Therapeutic role of carnitine**

There have been two excellent recent reviews summarizing human and rodent studies of carnitine supplementation (Zammit et al. 2009; Ringseis et al. 2012). Consequently, we provide a brief overview, but then focus on several key studies in relation to the lipotoxicity and mitochondrial overload hypotheses. For a comprehensive listing with specific focus on glucose homeostasis, the reader is directed to Ringseis et al. (2012). In Zammit et al., there is a slightly broader focus, and the significance of variant isoforms of carnitine shuttle enzymes with respect to metabolism is briefly discussed (Zammit et al. 2009).

**Overview of carnitine supplementation studies**

In nine animal studies, overall glucose tolerance was improved in all cases (Ringseis et al. 2012). The improvements were clearly dependent upon the insulin sensitivity of the animal, with improved glucose tolerance in
diabetic but not control mice (Ringseis et al. 2012). In 11 of 16 studies of humans with metabolic disorders, carnitine supplementation improved glucose tolerance, measured by either fasting glucose, fasting insulin, area under the curve (AUC) for glucose, AUC for insulin, glucose oxidation rate, or homeostatic model assessment of insulin resistance (HOMA-IR) (Ringseis et al. 2012). Two of the positive studies found that carnitine increased the efficacy of an antiobesity drug, but did not include a carnitine-only group (Derosa et al. 2003, 2010, 2011a, 2011b). Of the studies where carnitine did not change glucose tolerance, one study was characterized by only three male and three female subjects per group, and procedural difficulties resulting in a 30% increase in control group M-value during the 4-week trial (Gonzalez-Ortiz et al. 2008). The Bowyer study compared healthy and home parenteral nutrition subjects, but was woefully underpowered at four per group (Bowyer et al. 1989). The Galloway study had eight subjects per group, and found improvements to glucose disposal in lean, but not obese, subjects (Galloway et al. 2011). Bloomer found slight improvements to HOMA and AUC for glucose (Bloomer et al. 2009). In the Liang and Derosa studies, circulating lipids, but not fasting glucose, were lowered (Liang 1998; Derosa et al. 2003). In five of six studies of carnitine supplementation in healthy individuals, positive results on glucose tolerance were observed. The one negative study, as mentioned above, consisted of four subjects, raising doubts about any conclusions drawn (Bowyer et al. 1989). In fact, we used a power analysis (using source data for the precision of repeat measures of glucose disposal rate [GDR] from euglycemic-hyperinsulinemic clamp data collected at Pennington Biomedical Research Center) and set the target power at 80% and the significance level at 0.05. We calculated approximately 15 subjects/group were needed to detect a 25% improvement in GDR. De Gaetano et al. (1999) found increased glucose oxidation in healthy subjects. In contrast, both Ferrannini and Stephens found that enhanced glucose disposal was the result of increased glycogen storage in healthy individuals (Ferrannini et al. 1988; Stephens et al. 2006, 2007). Mingrone et al. (1999) found increased whole body glucose uptake and storage in both healthy and T2D subjects with carnitine supplementation, but glucose oxidation was increased only in the T2D group. Similarly, in diabetic subjects, Capaldo et al. (1991) found that increased glucose disposal was accompanied by decreased lactate levels, perhaps reflecting an increase in pyruvate oxidation. These studies encompass a wide range of measurement parameters, metabolic disorders, and treatment regimens. The current fragmentary nature of carnitine supplementation studies makes it difficult to draw definitive conclusions due to the wide variability in dosing, route of administration, and severity of metabolic dysfunction. The mechanistic data below provide strong rationale for further, more systematic clinical trials.
Mechanisms of carnitine’s effects in skeletal muscle

Several studies have explored the effects of carnitine and genetic manipulation of the carnitine shuttle system on glucose disposal in skeletal muscle. This is a logical focus since approximately 95% of the body’s total carnitine pool resides in muscle (Bhuiyan et al. 1988). The skeletal muscle also accounts for 80–90% of insulin-stimulated glucose disposal (DeFronzo et al. 1985). Consequently, whole body carnitine supplementation and knockout/overexpression strategies have often focused analysis on skeletal muscle effects, and these studies are included here.

Increased FAO

The lipotoxicity theory predicts that enhanced fat oxidation would improve insulin sensitivity by decreasing lipid accumulation and formation of DAGs and ceramides. Direct overexpression of CPT-1 has been used as a tool to increase FAO and decrease IMCL. Moderate increases in fat oxidation and improved insulin-stimulated glucose uptake have been observed following adenoviral or electroporation-mediated overexpression of CPT-1 in cultured myocytes and rat skeletal muscle (Perdomo et al. 2004; Sebastian et al. 2007; Bruce et al. 2009; Henique et al. 2010). Despite the many advances of the electroporation technique, there are two primary concerns raised with these studies: poor transfection efficiencies that may not yield uniform overexpression, and overexpression-induced changes to the proton conductance of the inner membrane that increase energy demand (Cadenas et al. 2002; Muoio and Neufer 2012). Other methods have also been used to increase FAO by indirectly increasing CPT-1 activity. Knockdown of acetyl-CoA carboxylase-2 (ACC-2), theoretically reducing concentrations of the CPT-1 inhibitor malonyl-CoA, does lead to increased FAO (Abu-Elheiga et al. 2001). When whole body energy expenditure is increased by enhanced oxidation of both fat and glucose, these mice maintain glucose tolerance and muscle insulin sensitivity (Abu-Elheiga et al. 2003; Choi et al. 2007). In other laboratories, ACC muscle and whole body knockouts still have increased fat oxidation, but reduced glucose oxidation and equivalent energy expenditure; however, glucose tolerance is unchanged from controls (Hoehn et al. 2010; Olson et al. 2010). Increased glucose tolerance and insulin sensitivity result in ACC–/– mice only when increased FAO is accompanied by increased energy demand and expenditure. Overall, these studies highlight the critical necessity of determining if enhanced FAO per se is beneficial to insulin sensitivity, or also requires increased energy expenditure.
Decreased FAO

If mitochondrial oversupply and resultant incomplete oxidation play an important role in development of insulin resistance, then manipulations to the carnitine shuttle that decrease fatty acid flux would be expected to play a protective role. The classic approach to decrease FAO is use of the CPT-1 inhibitors, etomoxir and oxafenicine. In both rodent and T2D human studies, CPT-1 inhibition increases glucose oxidation and reduces circulating glucose and insulin levels (Barnett et al. 1992; Hubinger et al. 1997; Deems et al. 1998; Dobbins et al. 2001; Timmers et al. 2012). In two studies, insulin sensitivity was not improved (Hubinger et al. 1997; Dobbins et al. 2001); however, Timmers et al. (2012) found improved insulin sensitivity in both human and rodent subjects despite increased DAG levels. CPT-1 inhibition also restored glucose tolerance in the muscle-specific PPARα overexpressing mouse, despite pronounced IMCL (Finck et al. 2005). Timmers et al. (2012) noted an increase in phosphorylation of the energy-deficit sensor AMPK, again highlighting that energy balance may be a key determinant of response. Others used a genetic approach to circumvent this concern and restrict β-oxidation specifically in muscle. By knockdown of malonyl-CoA decarboxylase, CPT-1 inhibition is achieved by increasing the endogenous concentration of malonyl-CoA (Koves et al. 2008). These mice maintain glucose and insulin tolerance despite challenge by a high-fat diet, in line with the theory that preventing mitochondrial lipid overload can preserve insulin sensitivity (Koves et al. 2008). One caveat of this approach is the lack of tissue specificity of these inhibitors as long-term, untargeted CPT-1 inhibition in the whole organism leads to hepatic steatosis (Vickers et al. 2006). However, a recent study using oxafenicine to specifically target cardiac and skeletal muscles also improved insulin sensitivity (Keung et al. 2013).

Reduced mitochondrial load

Carnitine shuttle manipulation can act not only to increase or decrease FAO and lipid access to mitochondria, but also to facilitate mitochondrial efflux of excess fat-derived acyl and both fat- and carbohydrate-derived acetyl groups via carnitine acetyltransferase (CrAT) (Muoio et al. 2012). Indeed, while a positive correlation exists between acetyl-CoA and free carnitine levels, no such correlation exists between long-chain acyl-CoAs and free carnitine, suggesting that augmenting carnitine concentration is more likely to alter CrAT activity than CPT-1 activity (Noland et al. 2009). As evident by the results discussed below, a prime feature of carnitine supplementation is enhanced glucose, rather than fat, oxidation, and reduced accumulation of β-oxidation intermediates.
An early study in KK-Ay mice with oral carnitine supplementation lowered blood glucose and improved glucose tolerance (Yoshikawa et al. 2003). Power et al. examined the impact of oral carnitine supplementation in severely insulin-resistant BAP-agouti, outbred insulin-resistant, and moderately insulin-resistant 45% high-fat-fed C57BL/6J mice (Power et al. 2007). In all cases, carnitine supplementation improved insulin tolerance and reduced basal blood glucose. Indirect calorimetric analysis of the BAP-agouti group showed both higher basal carbohydrate oxidation and an enhanced ability to increase carbohydrate oxidation in response to insulin. Enhanced carbohydrate oxidation and dramatically elevated plasma and urinary acetylcarnitine suggested that carnitine supplementation enabled efflux of excess acetyl-CoA, relieving PDH inhibition (Power et al. 2007; Mynatt 2009). This prediction was supported by carnitine stimulation of PDH activity in muscle homogenates (Power et al. 2007) and mitochondria (Uziel et al. 1988). Several studies have now provided data further strengthening the hypothesis that carnitine supplementation relieves mitochondrial lipid overload by enhanced efflux of excess mitochondrial acyl groups, improving metabolic flexibility and glucose utilization. Koves et al. (2005, 2008) found that diet-induced obese mice were characterized by enhanced incomplete β-oxidation, impaired substrate switching, accumulation of partially oxidized acylcarnitine species, and depletion of TCA cycle intermediates. Carnitine addition to isolated mitochondria increases efflux of catabolic and TCA cycle intermediates, increases membrane potential and oxygen consumption, and influences reactive oxygen species (ROS) production (Seifert et al. 2010a, 2010b). To determine whether acylcarnitine accumulation might reflect depletion of free carnitine under conditions of mitochondrial oversupply, a comprehensive analysis of carnitine status, mitochondrial function, and glucose tolerance was performed in rodent models of obesity, diabetes, and aging (Noland et al. 2009). In multiple models of insulin resistance, free muscle carnitine levels were lowered; however, plasma and urinary carnitine levels were unchanged (Noland et al. 2009). These results were subsequently confirmed in an independent study (Ringseis et al. 2011). Carnitine supplementation restored free carnitine content in tissues and improved glucose tolerance and HOMA index without altering basal insulin or glucose. Most notably, carnitine supplementation reversed the high-fat-mediated imbalance in incomplete:complete fat oxidation, and restored pyruvate-mediated inhibition of FAO, but did not alter PDH activity ex vivo (Noland et al. 2009). Urinary and plasma acylcarnitine levels were increased by carnitine supplementation, but did not substantially change in tissues, again suggesting a major role in efflux and excretion of excess acyl groups by the carnitine acetyltransferase (CrAT) enzyme (Noland et al. 2009). CrAT expression was unchanged by diet or age in rodents; however, there was a strong positive correlation between plasma acetylcarnitine and muscle
free carnitine levels, suggesting that even small changes to carnitine concentrations could influence CrAT activity (Noland et al. 2009). This conclusion is supported by in vitro kinetic assays (Chase 1967; Bhuiyan et al. 1988). Furthermore, accumulation of long-chain acylcarnitines, in addition to depleting free carnitine, may directly inhibit CrAT (Chase 1967; Huckle and Tamblyn 1983; Muoio et al. 2012). These studies prompted generation of a muscle-specific CrAT knockout mouse to definitively assess the role of CrAT in mitochondrial efflux of excess short-chain acyl groups. These studies were complemented by the use of primary human myocytes and a human carnitine supplementation study (Muoio et al. 2012). CrAT knockout mice have reduced insulin and glucose tolerance, and reduced metabolic flexibility, as indicated by reduced stimulation of whole body glucose oxidation during the fasted-to-fed transition or in response to insulin (Muoio et al. 2012). However, insulin-stimulated phosphorylation of both Akt and GSK-3B was similar to that of controls, suggesting the possibility of an alternative mechanism underlying their glucose intolerance. Substrate competition was clearly evident since carnitine was unable to stimulate PDH activity in CrAT-null mitochondria, and pyruvate-mediated inhibition of FAO was blunted (Muoio et al. 2012). In a clinical pilot trial of subjects with modest hyperglycemia, carnitine supplementation increased circulating free and acetylcarnitine, and lowered basal plasma insulin and glucose in insulin-resistant, but not insulin-sensitive, subjects (Muoio et al. 2012). Muscle homogenates from insulin-resistant subjects had reduced nutrient-induced substrate switching that was improved by carnitine supplementation (Muoio et al. 2012). To date, this is the only clinical trial to test carnitine-mediated efflux of excess acyl groups in relation to glucose tolerance, and may help to understand why carnitine supplementation seems more effective in glucose-intolerant than healthy subjects (Muoio et al. 2012).

Taken together, the above data suggest that carnitine supplementation is likely to exert therapeutic effects on insulin resistance not through enhanced FAO, but rather through facilitating efflux of excess partially oxidized acyl groups within the mitochondria. Since oxidation of both carbohydrate and fat converges at production of acetyl-CoA, carnitine supplementation is likely to be beneficial in cases of nutrient oversupply, regardless of nutrient source. This conclusion, however, is valid only if the supply of carnitine is limiting for acyl group efflux, but not for fatty acid mitochondrial import. Carnitine is synthesized endogenously at rates dependent upon both exogenous carnitine uptake and availability of cofactors including vitamins B6 and C, as well as iron. The major dietary source of carnitine coincides with a major dietary source of fat, namely, meat and dairy products. Thus, depending on diet, both carnitine levels and requirements may vary. In the next section, we discuss evidence for carnitine insufficiency in different populations.
Carnitine insufficiency as it relates to insulin resistance and type 2 diabetes

A secondary carnitine deficiency is often detected in patients presenting with inborn errors of carbohydrate, protein, or lipid metabolism. In fact, the link between alterations in substrate metabolism and carnitine status is so strong that, even in the absence of inborn errors in metabolism, insufficient carnitine levels are often described in diseases where profound changes in substrate metabolism are evident. This appears to be the case in insulin resistance and type 2 diabetes mellitus, and the link between these metabolic diseases and carnitine will be the topic of discussion in this section.

Circulating carnitine levels in healthy adult humans are 40 µM in females and 50 µM in males. Secondary carnitine deficiency has been defined as occurring when plasma carnitine levels fall below 20 µM (Winter et al. 1987), and humans with type 1 diabetes generally fall into this category (Cederblad et al. 1982; Winter et al. 1989; Pregant et al. 1991; Coker et al. 2002; Mamoulakis et al. 2004). Alternatively, those diagnosed with insulin resistance or type 2 diabetes largely do not meet these criteria. These individuals are often reported to exhibit 25–35% lower circulating free carnitine levels than normal (Okuda et al. 1987; Tamamogullari et al. 1999; Poorabbas et al. 2007; Kilicli et al. 2010), but differences are not always observed (Hoppel and Gennuth 1980; Adams et al. 2009). This raises an important issue concerning the reliability of using circulating free carnitine levels as a sole indicator of carnitine insufficiency, as there are instances where carnitine deficiency is not associated with alterations in circulating carnitine levels. Indeed, a myopathic form of carnitine deficiency has been described where carnitine levels are diminished in skeletal muscle, but unaltered in circulation. Since >95% of total body carnitine reserves are found within the skeletal muscle (Brass 1995), and this tissue is intimately involved in the pathophysiology of insulin resistance and type 2 diabetes, it is logical that investigation of this compartment may yield more conclusive insight into the prevalence of carnitine insufficiency in these metabolic diseases. Unfortunately, there are currently no studies providing direct evidence to link muscle carnitine levels with diabetes in humans; however, we can gather some insight from rodent studies. Accordingly, studies probing skeletal muscle in rodents unveil consistently lower (20–30%) free carnitine levels in various models of insulin resistance (genetic obesity, high-fat diet, and aging), and these decreases in free carnitine correlate with increases in the severity of insulin resistance (Noland et al. 2009). Moreover, the ability to maintain normal free carnitine levels when provided a high-fat diet was associated with a phenotype that was protected from the development of lipid-induced insulin resistance (Noland et al. 2009). Based upon this collective
evidence it seems that even this somewhat subtle 20–30% decrease in free carnitine observed in plasma or skeletal muscle of insulin-resistant and type 2 diabetic subjects likely represents a clinically relevant form of carnitine insufficiency. Causative factors leading to a carnitine-insufficient state in insulin resistance or type 2 diabetes remain poorly defined, but there are several possibilities.

**Sequestration of carnitine in the acylcarnitine pool**

Insulin-resistant and type 2 diabetic individuals have difficulty utilizing glucose as metabolic fuel, which often coincides with a hyperlipidemic environment. Lipids not only limit glucose utilization by serving as a competing substrate (Randle cycle—discussed in detail above), but various lipid intermediates have been shown to directly impede the insulin signaling cascade (reviewed in Samuel and Shulman 2012). Due to the essential role of carnitine to facilitate lipid entry into the mitochondrial matrix, speculation arose that diminished free carnitine limits FAO and thus contributes to accumulation of excess lipids and promotes worsening of the metabolic syndrome, which would be consistent with the phenotype observed in primary carnitine deficiency. Interestingly, however, this does not seem to hold true in the insulin-resistant/type 2 diabetic populations for a variety of reasons. First, administration of carnitine to individuals suffering from primary carnitine deficiency increases FAO, whereas this intervention actually promotes glucose (not lipid) metabolism in obese, insulin-resistant subjects (Capaldo et al. 1991; Mingrone et al. 1999; Power et al. 2007; Noland et al. 2009). Second, emerging evidence actually indicates that interventions designed to limit mitochondrial fatty acid entry have therapeutic effects in these individuals, rather than exacerbating insulin resistance (Koves et al. 2008; Timmers et al. 2011; Keung et al. 2013). Finally, due to the severely limited carnitine supply in individuals with primary carnitine deficiency, there are very low levels of esterified acylcarnitines present. In stark contrast, obese insulin-resistant individuals have elevated acylcarnitine accrual in skeletal muscle and plasma, indicating a heightened reserve of substrates available for immediate import into the mitochondrial matrix (Inokuchi et al. 1995; Koves et al. 2008; Adams et al. 2009; Noland et al. 2009; Ha et al. 2012). Based upon these findings it seems that the heightened acylcarnitine accumulation in obese, insulin-resistant individuals is simply a reflection of the hyperlipidemic environment. As a result, it is likely that the diminished free carnitine levels detected in this population are at least partially attributable to the fact that more of the carnitine stores are being sequestered in the acylcarnitine pool.
Urinary carnitine excretion

The kidney plays a vital role in maintenance of total body carnitine levels and is capable of rapidly and efficiently adjusting its rate of reabsorption to achieve this feat. Renal reabsorption of carnitine is accomplished by the action of organic cation transporter 2 (OCTN2), which is a chief reason as to why primary carnitine deficiency (a recessive autosomal defect in OCTN2) results in such severe carnitine depletion (<10% of normal levels). Unfortunately, whether alterations in renal reabsorption play a role in the carnitine insufficiency described in insulin resistance and type 2 diabetes is less clear. On one hand, some studies indicate that high-fat diets that induce insulin resistance do promote increased urinary excretion of free carnitine and acylcarnitines, and that people with type 2 diabetes exhibit heightened medium- and long-chain acylcarnitines in the urine (Cederblad 1987; Moder et al. 2003). Additionally, when placed on a carnitine-restricted diet, lean humans respond by limiting carnitine excretion in the urine, whereas this response is blunted in obese subjects (Hoppel and Gennuth 1980). These data suggest obese individuals may be more susceptible to developing carnitine deficiency if levels provided in the diet are low. On the other hand, separate studies provide evidence indicating there is either no change or decreased elimination of carnitine or acylcarnitines in the urine of insulin-resistant or type 2 diabetic subjects (De Palo et al. 1981; Morabito et al. 1994; Noland et al. 2009). However, since carnitine insufficiency is already present in these subjects, it is difficult to discern whether these results are a true reflection of renal function, or whether carnitine excretion simply represents the carnitine pool. Due to these conflicting results, further mechanistic studies are warranted in order to determine whether alterations in renal carnitine reabsorption truly contribute to the carnitine insufficiency observed in insulin-resistant and type 2 diabetic individuals.

Potential impairments in carnitine biosynthesis

Carnitine is found in high amounts in red meat and in moderate amounts in various dairy products (Ferreira et al. 1997). As a result, omnivorous humans obtain roughly 75% of their carnitine requirements through the diet, while the remaining 25% is produced through endogenous biosynthesis (Steiber et al. 2004). Despite the fact that the carnitine biosynthetic pathway has been quite well described (reviewed in Vaz and Wanders 2002), the involvement of this system in insulin resistance and type 2 diabetes remains virtually unexplored. PPARα agonists induce expression of carnitine biosynthetic genes resulting in increased carnitine production (Paul et al. 1986; Koch et al. 2008), while PPARα deletion decreases carnitine biosynthetic genes and significantly diminishes carnitine reserves.
van Vlies et al. 2007; Makowski et al. 2009). These data indirectly suggest these genes are lipid responsive. During the progression of lipid-induced insulin resistance it is common for PPAR-responsive genes to be acutely induced in order to combat the hyperlipidemic environment (reviewed in Holloszy 2009). However, substantial evidence indicates that many lipid-responsive genes are diminished in human cases of established insulin resistance and noninsulin-dependent diabetes mellitus (NIDDM), suggesting this response begins to fail as the disease progresses (Jans et al. 2011). In agreement with this assertion, rats receiving extended exposure to a high-fat diet displayed diminished expression of genes involved in carnitine biosynthesis, which may have contributed to the reduced free carnitine levels observed in these animals (Noland et al. 2009). Unfortunately, a causal link was not established in this study and similar reports have yet to surface in humans.

Another factor that could contribute to diminished carnitine biosynthesis in type 2 diabetics relates to findings indicating vitamin C (ascorbic acid) levels are diminished by nearly 30% in these individuals (Will and Byers 1996; Sargeant et al. 2000). Vitamin C is an important cofactor for enzymes involved in carnitine biosynthesis, and vitamin C depletion has been found to decrease carnitine biosynthesis (Hughes et al. 1980; Nelson et al. 1981; Sandor et al. 1983; Dunn et al. 1984; Thoma and Henderson 1984; Rebouche 1991). Unfortunately, extrapolation of these findings toward human type 2 diabetes remains difficult because the aforementioned studies were performed in scorbutic models and thus invoked a more severe decrease in vitamin C than is observed in diabetics. Based upon the above observations it is feasible that carnitine biosynthesis is diminished in a state of advanced insulin resistance, but until specific studies are performed in diabetic humans to provide conclusive evidence this remains speculative.

**Intestinal carnitine absorption**

As mentioned earlier, roughly 75% of the total carnitine content in omnivorous humans is obtained through the diet. With this in mind, the intestinal absorption of carnitine has the potential to play a significant role in fluctuations in carnitine balance. L-Carnitine is absorbed in the small intestine primarily through the high-affinity OCTN2 transporter, while the low-affinity amino acid transporter ATB(0,+) is also involved to a lesser degree (Nakanishi et al. 2001; Duran et al. 2002; Elimrani et al. 2003; Hatanaka et al. 2004; Kato et al. 2006). Administration of clofibrate, which is often used as an antidiabetic agent, increases OCTN2 expression in the small intestine and leads to increased carnitine absorption (Ringseis et al. 2007, 2008a, 2008b). Individuals afflicted with inflammatory bowel disease exhibit diminished intestinal OCTN2 expression and
carnitine absorption and tend to have a greater frequency of the metabolic syndrome (D’Argenio et al. 2006; Yorulmaz et al. 2011). These data provide at least anecdotal evidence linking diminished carnitine absorption to development of the metabolic syndrome. Unfortunately, however, there are currently no studies directly establishing this link, and this remains an issue that needs to be addressed.

It is important to note, however, that provision of supplemental carnitine rapidly restores circulating and tissue carnitine to normal or above-normal levels in nearly all cases of carnitine deficiency, indicating that potential defects in intestinal carnitine absorption can likely be overcome by increasing dietary intake. With this in mind, it is important to remember that the amount of carnitine absorbed through the small intestine is dependent upon the dietary intake of carnitine; therefore, consideration must be given to the possibility that insufficient carnitine intake may contribute to the pathophysiology of insulin resistance and type 2 diabetes. Carnitine content is highest in meat products (particularly red meat), is moderately present in dairy products, and is very low in fruits and vegetables. Based upon this distribution, it appears foods derived from mammalian sources that contain high lipid content enjoy a natural enrichment in carnitine. This is likely of great benefit due to the essential role carnitine plays in facilitating FAO. Unfortunately, not all high-fat foods can boast high carnitine levels. Moreover, it is likely that hypercaloric diets that contain low carnitine levels are the most commonly associated with an obesogenic lifestyle. In support, recent studies indicate that provision of supplemental carnitine at the onset of an obesogenic diet prevents the development of insulin resistance in rodents (Power et al. 2007; Rajasekar and Anuradha 2007a, 2007b; Rajasekar et al. 2007). Ultimately, however, more definitive research is needed to define whether carnitine intake or intestinal absorption is mechanistically linked to the development of the metabolic syndrome.

Maternal health and nutrition

Substantial evidence exists indicating maternal health and nutrition have a significant impact on fetal development, and that maternal overnutrition can transfer a metabolic imprint to the offspring that predisposes them to the development of the metabolic syndrome (reviewed in Rooney and Ozanne 2011). Carnitine levels in breast milk are relatively high, and carnitine is added to infant formula, emphasizing its importance in the health of newborns. With this in mind, the question arises as to whether alterations in maternal carnitine status are associated with fetal growth and development, and how this may parlay into health of the offspring. While evidence remains sparse, there are findings indicating that carnitine status is compromised in women presenting with gestational
diabetes (Gunter et al. 2002; Pappa et al. 2007). Additionally, provision of supplemental carnitine during pregnancy helped mitigate large increases in plasma-free fatty acids, which are thought to be primary drivers of gestational diabetes. Unfortunately, little evidence exists regarding the impact of this form of carnitine insufficiency on fetal health in the context of gestational diabetes, but perhaps we can glean understanding from other models. For example, provision of pivalate to rodents during pregnancy and lactation induces mild carnitine insufficiency in the mother. Pups born to these mothers exhibited diminished carnitine in liver, skeletal muscle, and plasma that was associated with elevated circulating ketones and excess ectopic lipid accumulation in liver and skeletal muscle (Ricciolini et al. 2001). Similar findings were shown in an experimental rodent model of type 1 diabetes, as destruction of pancreatic β-cells via streptozotocin administration resulted in decreased maternal carnitine levels and the dams gave birth to pups that had similarly reduced carnitine levels in the heart (Akisu et al. 2002). These findings suggest maternal carnitine insufficiency may predispose their offspring to the development of the metabolic syndrome.

**Potential problems with carnitine deficiency**

Due to its essential role in facilitating fatty acid transport into the mitochondrial matrix, carnitine insufficiency or deficiency would predictably limit FAO. However, carnitine is involved in myriad biological processes that are linked to metabolic health, and inadequate carnitine levels would be predicted to compromise these as well. Principally, carnitine has been described to have antioxidant-like properties, and diabetes is a condition known to have elevated oxidative stress. Heightened oxidative stress has been linked to impaired substrate metabolism, impaired neural function contributing to diabetic polyneuropathy and retinopathy, and diminished wound healing. These complications lead to some of the most catastrophic outcomes associated with these metabolic diseases and are among the most costly in terms of treatment options. Unfortunately, a consensus has not been reached regarding the association of diabetic complications with carnitine status. Early studies found those with more diabetic complications had lower carnitine levels (Tamamogullari et al. 1999; Poorabbas et al. 2007); however, a recent study observed that diabetics with the lowest carnitine levels did not have greater incidence of complications, and those with the highest carnitine levels did not have reduced frequency of complications (Liepinsh et al. 2012). Regardless, there is a fair amount of research indicating that provision of supplemental carnitine to insulin-resistant or diabetic subjects not only improves glucose homeostasis, but also may be beneficial in mitigating diabetic complications.
Summary and prospective studies

Based on the results of studies that have elegantly evaluated the role of both insulin secretion and insulin resistance, it is now well established that type 2 diabetes is a progressive disease. The current nonpharmacological management for patients with type 2 diabetes utilizes nutrition therapy and enhanced physical activity as cornerstones of treatment. When that approach fails to achieve glycemic goals, the patient is traditionally advanced to strategies to improve glucose control by pharmacological means. However, because of the widespread use of dietary supplements by the general public, supplementation with the use of agents such as carnitine that may effectively increase insulin sensitivity or restore deficient carnitine levels represents a very attractive and novel approach for adjunctive therapy of diabetes. Critical well-powered studies using state-of-the-art methodologies in randomized, parallel-arm, double-blinded, placebo-controlled clinical trials are needed to test the carnitine hypothesis in a definitive manner and to comprehensively evaluate the potential mechanism(s) by which carnitine affects insulin action, lipid and glucose metabolism, and mitochondrial function in humans. Included in the design should be plasma and tissue samples for metabolomic analysis at baseline and end of study to possibly identify individuals in which carnitine supplementation will be most efficacious.

Completion of these projects may also have public health significance. There is a paucity of data on the mechanism and efficacy of carnitine supplementation in prediabetes (metabolic syndrome) and in subjects with type 2 diabetes. Positive results from such carefully designed supplementation studies would allow recommendation for a relatively inexpensive adjunctive treatment such as carnitine for both the obese, insulin-resistant diabetic and the individual with diabetes. The results of these studies will provide the critical data necessary to generate recommendations for or against routine use of carnitine supplementation and whether a health claim can be made.

References


Peripheral arterial disease (PAD)—a clinical manifestation of underlying aortoiliac and leg atherosclerosis—is characterized by different degrees of arterial stenosis or obstruction, with progressive reduction in distal perfusion pressure and blood flow. Clinically, patients with PAD can present without symptoms (asyPAD), with intermittent claudication (IC) until critical leg ischemia (CLI), or with persistent resting pain with or without ischemic skin lesions (necrosis and gangrene).

Intermittent claudication—the main symptom of PAD—is defined as cramping leg pain (in the calf, thigh, or buttock) while or after climbing one or two flights of stairs, or during walking, which disappears after resting [1].
IC affects about 12% of adults and 20% of individuals over 70 years of age [2]. It is associated with increased cardiovascular (CV) and cerebrovascular morbidity (5% of nonfatal events in 5 years), and a six-times-greater risk for death (30% in 5 years) than the general population [3–8]. Risk of progression to more advanced stages of PAD is about 25% in 5 years [4, 9].

The stage of severe claudication (symptoms include walking less than 100 m or climbing less than one flight of stairs) is associated with a 3-year mortality rate of 20% and an extremely high risk of the affected limb worsening; 40% of cases progress to CLI in 6–12 months and 35% require major amputation within 24 months [9, 10].

Different facets of intermittent claudication

The pathophysiology and clinic of IC have some peculiar facets [11]. The first is hemodynamics. In the 1970s, numerous studies defined the local hemodynamics of the lower limb with PAD, characterized by almost constant flow at rest in all stages of the disease, while the maximum postischemic flow (reactive hyperemia) decreases progressively, thus reducing the functional reserve [12]. However, if we consider the curve of the ankle pressure of a patient with PAD during a treadmill test [13], we observe a progressive decrease of the pressure, with gradual recovery after cessation of exercise. At the same time, there is a reduction in oxygen levels with increased acidosis [14–16], which peaks during the recovery period [17]. Indeed, the hemodynamics of an exercising PAD patient are not dissimilar to those observed in the experimental model of ischemia-reperfusion [18]. Subsequently, IC can be considered a spontaneous model of ischemia-reperfusion in vivo [19]. It is known that altered endothelial function is a component of ischemia-reperfusion syndrome, which is accompanied by a reduction in the vasodilating capacity and an increase in inflammation. Several studies have shown that during maximal exercise there is an increase of leukocytes and thromboxane levels [20], lipid peroxidation [21], and oxidized LDL [22], in addition to increased cytokine and adhesion molecule release in patients with PAD, impairing endothelium-dependent vasodilation (flow-mediated dilation [FMD]) [23]. The increase in inflammatory cytokines, particularly interleukin (IL) 6, is thought to be a key component of exercise-induced inflammation in PAD patients. Indeed, the claudicant patient, when compared to controls, has a higher level of circulating IL-6 at rest, which increases further during maximal exercise. Moreover, in severe claudication this trend continues to rise even postexercise [24].

These data confirm that IC is a true model of ischemia-reperfusion injury, with progressive worsening of endothelial function (EF). A study by our group has shown that EF, measured as FMD, is significantly impaired in two-thirds of claudicants, and worsens during maximal
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Exercise [25, 26]. Endothelial dysfunction triggers the process of evolution of the atherosclerotic plaque throughout the arterial tree (coronary, carotid, etc.). Under the inflammatory stimulus stable plaque becomes unstable, with high risk of erosion and rupture. This progression of atherosclerotic lesions offers an explanation for the reduced life expectancy of claudicants, and allows us to consider IC as a risk factor for cardiovascular events, both fatal and nonfatal.

Intermittent claudication as metabolic myopathy

The pain experienced by patients suffering from IC depends on the discrepancy between the oxygen required by skeletal muscle and the oxygen provided by the arterial supply. Indeed, walking capacity in mild to moderate claudication is directly correlated with oxygen delivery and inversely correlated with acidosis [19]. In severe claudication, the relationship with oxygen is reduced, while it increases, the inverse correlation, with acidosis [27], which likely reflects a chronic adaptation to hypoxia.

The structure of the skeletal muscle is characterized by three types of muscle fibers: type 1, oxidative, adapted for prolonged work; type 2B, glycolytic, adapted for faster activities, which fatigues faster; and type 2A, intermediate of type 1 and type 2B fibers. Claudicants lose type 2B fibers and have a higher prevalence of intermediated type 2A fibers [28], which can be read as a compensatory response to reduced glycolytic muscle fibers [29].

In addition, it should be considered that not only is skeletal muscle structurally in PAD patients, but there are also significant changes in muscle function. Studies using $^{31}$P nuclear magnetic resonance spectroscopy (MRS) have shown that mitochondrial function is significantly impaired in the muscle of claudicant patients [30, 31], and that this intrinsic mitochondrial impairment persists after revascularization, despite normalization of hemodynamic parameters [32, 33]. All these data support the concept of defective mitochondrial function as a pathophysiological component of PAD [34].

Normally, fats and carbohydrates are converted to acyl-coenzyme A (CoA) intermediates to be used in the Krebs cycle. In the case of mitochondrial dysfunction, reducing utilization of acyl-CoA leads to its accumulation. Increased acyl-CoA levels then lead to inhibition of the metabolic pathways (catabolism of lipids, proteins, and carbohydrates) involved in its production [35]. To prevent this adverse event, the carnitine system buffers the increased acyl-CoAs by collecting their acyl groups with increase of acyl carnitines in tissues and plasma [36]. Interestingly, claudicant patients have abnormally high levels of acyl carnitines in both skeletal muscles and blood (reflecting poor mitochondrial utilization and oxidation of acyl-CoA), and these levels correlate with the degree of walking impairment [37, 38].
Carnitine acts as a physiological buffer of acyl groups, where mitochondrial dysfunction in PAD patients causes an increase in carnitine utilization, and therefore in demand of carnitine, leading to a secondary deficit of carnitine, with a metabolic myopathy similar to the cardiomyopathy caused by the primary deficit of carnitine.

**Carnitine**

Carnitine is an endogenous quaternary amine that is synthesized in the liver and kidney [39]. Under normal metabolic conditions, skeletal and myocardial cells derive energy from the mitochondrial oxidation of fatty acids [40]. Carnitine plays a crucial role in transporting fatty acids, which are coupled with coenzyme A (CoA), from the cytosol into the mitochondrial matrix for oxidative metabolism [41]. Only the L-isomer of carnitine (L-carnitine) is physiologically active in the transport of fatty acids from the cytoplasm into the mitochondrial matrix. In addition, L-carnitine acts as a buffer of the acyl-CoA pool through the reversible transfer of acyl groups between CoA and carnitine [39]. In ischemic skeletal muscle, levels of free carnitine, acyl carnitine, and acetyl carnitine transferase activity are altered [39, 40].

Propionyl L-carnitine (PLC), the propionyl ester of L-carnitine, exhibits a high affinity for the muscle enzyme, carnitine acyl transferase, and as such readily converts into propionyl-CoA and free carnitine [42]. It was thought that PLC would improve muscle metabolism and stimulate oxidative phosphorylation by increasing cellular carnitine levels.

Numerous studies have provided insight into the mechanistic properties of PLC in PAD. Results from several preclinical studies in animal models of PAD have shown that PLC preserves carnitine levels in muscle tissue and confers vasoprotective effects such as:

- Reducing plasma extravasation during the PAD-induced inflammatory process [43]
- Modulation of endothelin-induced prostanoid release and increased prostaglandin synthesis to counteract vasoconstriction [44, 45]
- Reducing peripheral vascular resistance by means of a direct vasodilatory effect in musculocutaneous vascular beds [46]
- Increasing fatty acid oxidation and preservation of high-energy phosphate levels in vascular and muscular tissue [47]

Pharmacodynamic studies in patients with PAD showed that PLC facilitates fatty acid oxidation by increasing intracellular levels of L-carnitine [48, 49].

PLC provides protection from the effects of oxidative stress and inflammation in ischemic tissue endothelium by reducing the plasma
release of leukocyte-endothelial adhesion cells, of E-selectin, P-selectin, L-selectin, and other chemokines [50, 51], improving arteriolar function [52], and increasing arterial flow-mediated dilation (FMD) [51]. Moreover, PLC improved arteriolar function and reduced acidosis without affecting arterial inflow in a human model of ischemia-reperfusion [52].

**Clinical efficacy of L-propionyl carnitine supplementation**

The clinical efficacy of PLC in patients with IC has been derived from studies found in the literature quoted in MEDLINE and EMBASE. The literature searches were limited to humans, and studies involving less than 12 patients were excluded. The searches were supplemented by manual screening of reference lists of included studies.

Generally, across all trials, the included patients were adults, had various degrees of IC, and in most cases patients with type 2 diabetes were not excluded.

The clinical focus of most trials in PAD was the effect on walking capacity measured by walking distance or by the length of time the patient was able to walk, relative to baseline. Walking distance was measured by two parameters, initial claudication distance (ICD) or pain-free walking (PFW) distance, and the absolute claudication distance (ACD) or maximal walking distance (MWD). Other outcomes include the effect on hemorheological parameters, such as capillary perfusion, oxidative parameters, and the ankle/brachial index (ABI), which has been shown to correlate with the morbidity of arterial pathologies of other sites. In some trials, these outcomes were investigated in conjunction with quality of life [53].

**Effects on walking capacity**

In five large, randomized, double-blind, placebo-controlled studies [54–58], PLC treatment showed a significant improvement in ICD and ACD, with a remarkable increase in ACD and in maximal walking time. More profound results were found in the most compromised patients (baseline ACD ≤ 250 m) compared to patients with mild functional impairment (i.e., ACD > 250 m) [55].

Smaller studies with shorter treatment durations have confirmed a significant improvement in ACD following PLC treatment [59–61]. Furthermore, a 12-month comparison study with pentoxifylline again confirmed the efficacy of PLC, with significantly greater improvements in walking capacity following PLC administration than pentoxifylline administration [62].
Another small, placebo-controlled study involved 44 patients with moderate (ACD < 200 m, n = 22) or severe claudication (ACD < 100 m, n = 22) selected to receive a supervised physical training program (three times a week for 6 weeks, tailored at 60–70% aerobic exercise of the patient’s walking ability [63]). During the first 3 weeks, all patients undertook the standard physical training program. In the following weeks, before the daily training session, two subgroups of moderate claudication (M-CL) and severe claudication (S-CL) received a 250-ml intravenous saline infusion, whereas the other subgroups received 250 ml intravenous saline solution plus 600 mg propionyl L-carnitine (M-CL-PLC and S-CL-PLC, respectively) (Figure 8.1A); ACD was measured at the end of the third and sixth weeks [30]. The results of this study confirm the well-established role of supervised physical training in the treatment of patients with IC. ACD significantly increased in all patients (p < .001 vs. baseline) during the second phase of the study. However, the increase in ACD was higher among patients receiving PLC (Figure 8.1B). The addition of PLC to the supervised physical training program provided a significant increase of ACD in both moderate (+9.20%, p < .006) and severe (+20.90%, p < .0003) claudication, but the supplementation mostly benefited patients with the highest muscle metabolic deficiency [64]. The authors suggest that the use of PLC during an exercise training program should be recommended in patients with IC, and a cycle of PLC infusions could be advised in patients with severe claudication who cannot be included, for any reason, in an exercise training program.

![Study design diagram](image)

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<td><strong>Study design</strong></td>
<td>Baseline, intermediate, and final values of absolute claudication distance during physical training program, in patients with moderate claudication (M-CL) and severe claudication (S-CL) treated with (M-CL-LPC and S-CL-LPC) or without (M-CL and S-CL) levo-propionyl-carnitine.</td>
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**Figure 8.1** Levo-propionyl-carnitine improves the effectiveness of supervised physical training on the absolute claudication distance in patients with moderate (M-Cl) and severe (S-Cl) intermittent claudication. (From Andreozzi et al., Angiology 2008;59(1):84–89.)
Hemodynamic effects

Results on ABI are controversial. A significant increase in ABI was observed in several studies [55, 59–61, 65, 66], but other studies produced no significant differences. In addition, some of these studies also observed a reduced peripheral resistance measured by continuous wave Doppler of the lower limbs [60, 66]. However, the differences found were modest and clinically insignificant; they probably are the expression of an improvement in endothelial function rather than indicators of improved hemodynamics (increased inflow) [25].

Oxidative profile and endothelial function

PLC protects tissues from oxidative damage [67–69]. In a murine model of ischemia-reperfusion injury, PLC preserved endothelial function and decreased ischemia-related microvascular permeability [70]. A similar cytoprotective activity was also demonstrated in the human ischemia-reperfusion model with a significant reduction in acidosis after ischemic stress (Figure 8.2) [52]. PLC also plays an important role in membrane phospholipid fatty acid turnover and inhibits arachidonic acid accumulation in platelet phospholipids, platelet function, and oxidative stress [71].

The impact of PLC on oxidative profile has been evaluated in two studies in patients with chronic renal failure [65] or PAD associated with type 2 diabetes [58]. These studies showed positive effects on plasma markers of oxidation (malondialdehyde [MDA], 4-hydroxynonenal [4-HNE], and the nitrite/nitrate [NO\textsubscript{2}/NO\textsubscript{3}] ratio) after 12 months of treatment. This antioxi-

![Figure 8.2](image)

**Figure 8.2** Transcutaneous carbon dioxide tension (TcPCO\textsubscript{2}), representing the acidosis production, in 16 patients with intermittent claudication undergoing an ischemia-reperfusion test. Propionyl-L-carnitine reduces TcPCO\textsubscript{2} values at hypoxia and during hyperemia ($p = .001$ and $p = .0006$, respectively, vs. untreated condition). (From Andreozzi et al., *Clin Drug Invest* 2002;22(Suppl 1):15–21.)
PLC administration provided a significant protective effect against deterioration of the postexercise decrease in brachial artery FMD and exercise-induced increases in plasma concentrations of soluble vascular adhesion molecule-1 (sVCAM-1) in a study performed in 2006 involving patients with IC who had pronounced endothelial dysfunction after treadmill exercise [51].

In 2007, in a placebo-controlled, crossover study involving 21 patients with PAD who received PLC (6 g/day) and placebo for 7 days, FMD was significantly increased from baseline with PLC but not with placebo \((p < .001 \text{ vs. baseline})\). Similarly, compared with baseline, serum levels of \(\text{NO}_2/\text{NO}_3\) were significantly increased, and those of 8-hydroxy-2-deoxy-2-deoxyguanosine significantly decreased with propionyl L-carnitine but not placebo administration [72].

**Effects on quality of life**

PLC enhances most measures of quality of life (QoL) in patients with PAD relative to placebo [54]. Compared with placebo, PLC significantly improved QoL overall in patients with more severe IC \((\text{ACD} \leq 250 \text{ m})\) [55–57, 73]. Walking Impairment Questionnaire scores remained constant with 6 months placebo administration, but were significantly improved with PLC for walking speed \((p < .05)\), body pain, and improved health state \((p < .01)\) [55].

**Conclusive remarks**

Considering the pathophysiological features of metabolic myopathy of intermittent claudication and secondary carnitine deficiency that characterizes it, supplementation with PLC should always be considered in claudicant patients.

PLC, in fact, has been shown to correct the deficiency in carnitine, while significantly improving the walking ability and quality of life in patients with IC. For these reasons the documents TASCI [74] and TASCII [75] recommend the use of the PLC.

The recommended doses are, for oral therapy, two tablets of 500 mg twice a day, and for intravenous therapy, 300–600 mg per day in 250 ml of saline or dextrose, for 7–20 days.

Unfortunately, despite health-related quality of life being an important factor in the assessment of therapeutic products, the regulatory authorities of public health programs still do not consider the improvement of walking capacity a primary endpoint of treatment of IC.

Furthermore, considering the activities on oxidative stress and endothelial function, PLC could also have a role in slowing the progression of atherosclerosis.
Finally, these pharmacological properties, together with cytoprotective effects observed in models of ischemia-reperfusion injury, also would support a protective role of PLC in the treatment of critical limb ischemia, as already suggested by the results of open clinical studies carried out in patients with CLI and ischemic skin wound [76–79], in association with treatment with prostanoids.

For all of these reasons, PLC is an evidence-based therapy for patients with PAD.

Declaration of interest

Carnitine therapy has been developed in Italy by Sigma-Tau Pharmaceutical Company and launched under the trade name Dromos®. It is indicated for patients with peripheral arterial occlusive disorders and for exercise intolerance enhancement in patients with chronic congestive heart failure. Dromos is marketed in Italy by Biofutura Pharma S.p.A. (Milan, Italy).

Prof. G.M. Andreozzi, in the past, has formerly been a freelance scientific consultant and provided expert testimony for Biofutura Pharma. He did not receive a fee for this work.

References


Background/theoretical basis

Interest in L-carnitine as a potential ergogenic aid for sport performance spans over 30 years. During this time there have been many human and animal studies and reviews published. While interest in the topic has fluctuated alongside the positive and negative results of these studies, more recently, interest has increased again due to a number of positive observations on substrate metabolism in human exercise studies. The early studies conducted in the 1980s and 1990s examining carnitine ingestion and infusion typically investigated short-duration supplementation either from acute doses administered a few hours before exercise or from doses administered over 4 to 6 weeks of daily ingestion. Several of these early studies suggested an ergogenic effect of carnitine on variables related to sport and exercise performance, such as increased maximal aerobic capacity (VO$_{2\text{max}}$), increased estimates of whole body fat oxidation during exercise, reduced blood lactate accumulation during exercise, maintenance of muscle carnitine content with training, and improved recovery from exercise. However, these observations were countered by a similar number of studies demonstrating no beneficial effects on these markers of performance. The first section of this chapter will aim to provide an overview of these early observations and highlight the equivocal outcomes from the many studies.
The early studies demonstrating positive improvements in VO\textsubscript{2max} with carnitine ingestion were met with some skepticism as they could not be specifically defined by the supplementation duration, supplement dose, timing of intake prior to exercise, or mode of exercise. For example, Marconi et al. [1] demonstrated a 6\% increase in treadmill VO\textsubscript{2max} following 2 weeks of L-carnitine ingestion at a dose of 4 g/day, whereas Vecchiet et al. [2] demonstrated an increased cycle ergometer VO\textsubscript{2max} at only 60 minutes following the ingestion of an oral dose of 2 g of L-carnitine. The latter of these studies [2] was also subsequently criticized regarding the timing of L-carnitine intake and the possible magnitude of change in intramuscular total carnitine content (<1\%) [3]. This questioning of any potential effect through an action within skeletal muscle suggested that the observed ergogenic effects were either false positive observations or had occurred due to systemic actions of carnitine on other organs or tissues. The publication of studies that could not support these prior observations of increased VO\textsubscript{2max} (e.g., [4]) suggested that the initial observations may be due to false positive outcomes. However, these early studies did raise several important questions relating to the theoretical basis for improvements in VO\textsubscript{2max} with L-carnitine supplementation, and also provided the impetus for two different avenues of research: (1) studies based around skeletal muscle metabolic explanations and (2) studies based around systemic changes in plasma carnitine that could impact upon the function of other organs/vessels. These two paths of interest are still considered in current carnitine research, especially given the lengthy period (~6 months) now known to be required for alterations in human skeletal muscle carnitine content with oral ingestion [5].

The theoretical basis for any effect of L-carnitine on VO\textsubscript{2max} should consider the factors thought to limit maximal work capacity and oxygen delivery. Basic human exercise physiology research informs us that VO\textsubscript{2max} is limited mainly by cardiac output [6]. However, peripheral oxygen extraction is also an important component in the Fick equation, so adequate perfusion of contracting skeletal muscle through an increased capillary-to-muscle fiber ratio, and the ability to utilize oxygen in the periphery through increased mitochondrial density, also play a role. More recently, the central neural influences on contracting skeletal muscle have also been presented, and these probably should be considered when thinking about factors that limit maximal work capacity [7]. Thus, for L-carnitine to influence VO\textsubscript{2max} it must act on one or all of these potential regulatory points and overcome an existing limitation. Some research does support a role on promoting glucose oxidation and improving contractility in cardiac muscle [8, 9] and increased perfusion and oxidative capacity in skeletal muscle [10], but these observations have not been consistently observed in humans.
Despite the multiple sites of potential limitation to maximal exercise, the key focus of investigation by researchers examining ergogenic effects of L-carnitine has been on skeletal muscle substrate metabolism. Studies in the early 1980s demonstrated that carnitine content in a tissue was related to the capacity for fatty acid oxidation, and that the carnitine content appeared to reflect the optimal concentration required for maximal rates of fatty acid oxidation [11, 12]. Since then, research on fat metabolism has revealed several sites of regulation, including at the site of carnitine palmitoyl transferase I (CPT1) for transfer of activated fatty acids across the outer mitochondrial membrane. It is still a topic of considerable debate as to whether carnitine is limiting to CPT1 [13]. Other control points include: (1) regulation of lipolysis within adipose tissue and intramuscular triacylglycerol pools, (2) transport and delivery of circulating free fatty acids from adipose tissue stores to working skeletal muscle, (3) transport of fatty acids across the plasma membrane and within the cell cytosol to the outer mitochondrial membrane, and (4) the capacity for fatty acid oxidation by the β-oxidation pathway [14]. However, the contribution of each of these on the regulation of fat metabolism within contracting skeletal muscle is not fully understood. Despite this lack of knowledge, there is increasing evidence to suggest that fat oxidation is regulated by glycolytic flux and potentially a knock-on inhibition of CPT1. For example, during exercise at 50% VO\(_{2\text{max}}\), hyperglycemia (induced as a result of ingestion of glucose) increases glycolytic flux and reduces long-chain fatty acid oxidation [15], whereas medium-chain fatty acid oxidation is unaffected. This suggests an inhibition of fat oxidation at the level of CPT1, possibly by reduced availability of free carnitine through the buffering of excess acetyl CoA to form acetylcarnitine. Therefore, for carnitine to influence fat metabolism, its content in skeletal muscle must be raised, and the impact of high glycolytic flux on CPT1 activity reduced (or glycolytic flux itself reduced).

Early studies examining substrate use following L-carnitine supplementation revealed a similar confusing picture to those examining maximal aerobic capacity, i.e., some supportive of an effect on increased fat metabolism [16] and some showing no effect at all [17]. Other studies demonstrated reduced lactate accumulation during exercise and attributed this to increased activation of pyruvate dehydrogenase enzyme complex (PDC), or reduced flux through glycolysis as a result of increased reliance on fat metabolism [18, 19]. Interestingly, an increased activation of PDC would only be facilitated if greater free carnitine availability occurred within the mitochondrial matrix to enable greater production of acetylcarnitine to buffer excess acetyl CoA production. While this would reduce lactate accumulation through increased pyruvate entry into the mitochondria, this effect could then limit free carnitine availability for fatty acid transport at the level of CPT1. Aiding the maintenance of a high
glycolytic flux would not necessarily be considered beneficial to sustained endurance exercise capacity due to more rapid depletion of endogenous muscle glycogen stores. In contrast, high glycolytic flux with a reduced accumulation of lactate could be considered desirable for high-intensity short-duration exercise tasks. The key factors influencing carbohydrate metabolism are currently thought to include substrate delivery (perfusion of the muscle), glucose transport capacity, glycogen phosphorylase activity (in turn influenced by muscle glycogen content and free inorganic phosphate), and downstream feedback controls [20]. Some, but not all, of these areas were investigated in early studies, but more work on this has been completed recently and will be discussed later in the chapter.

As research on the potential ergogenic effects of carnitine increased, considerable debate revolved around the usual issues: Are changes in muscle total carnitine or free carnitine possible following oral ingestion of L-carnitine? If so, is 7 days or 4 weeks enough? In addition, there was still no consensus as to whether free carnitine could be limiting, or whether total carnitine content of skeletal muscle could be depleted substantially during exercise, or through prolonged periods of exercise training. Lennon et al. [21] reported a reduction in total muscle carnitine with an increase in plasma acylcarnitine following 40 minutes of exercise at moderate intensity (55% VO$_{2\text{max}}$), but a reduction in total carnitine content of skeletal muscle was not observed following 90 minutes of exercise at 50% of VO$_{2\text{max}}$ in a study by Carlin et al. [22]. Decombaz et al. [23] went on to show that total muscle carnitine content did not change with over 13 hours of strenuous exercise in cross-country skiers, and concluded that training would not likely impair total muscle carnitine content. However, Arenas et al. [24] demonstrated that 6 weeks of exercise training resulted in a small decline in total carnitine content of skeletal muscle, and that this decline could be prevented by L-carnitine ingestion. These observations of Arenas et al. [24] were the first to indicate that L-carnitine content of skeletal muscle could be increased (or at least a decline prevented) through prolonged oral carnitine ingestion, but the study also raised a new dilemma concerning carnitine homeostasis: if athletes could lose carnitine from skeletal muscle with exercise training, then why is the loss not countered by increased renal reabsorption of carnitine or compensated for by an increased energy intake of foods containing the necessary precursors for endogenous synthesis of carnitine [25, 26]? If a loss of carnitine from skeletal muscle did occur with training, would this not be contrary to the observations of an increased reliance on fat as a fuel observed following a period of endurance training? Dietary intake studies of adults (nonvegetarians) previously had suggested that an increase in energy intake would likely be sufficient to provide enough carnitine and carnitine precursors to support maintenance of tissue and plasma carnitine homeostasis [27], and this was later confirmed, at least for carnitine intake.
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and plasma carnitine status, in athletes [28]. Therefore, the debate around potential loss of carnitine with training is still unresolved. The question then becomes whether changes in free carnitine content or the ratio of free carnitine to acylcarnitine could impact upon metabolism in the absence of a decline in muscle total carnitine content. This question was at least partly answered by studies from van Loon et al. [29] and Roepstorff et al. [30], in which the authors demonstrated that acute exercise could reduce the muscle free carnitine content. The decline in free carnitine was dependent upon the intensity of exercise and fell below resting concentrations at intensities above about 60% of VO$_{2\text{max}}$ (similar to the intensity at which whole body fat oxidation begins to decline).

One final area of early carnitine work investigated the role of carnitine supplementation in postexercise muscle damage and recovery [31–34]. These studies hypothesized that carnitine could impact upon damage or recovery through an enhanced blood flow response to contracting muscle during exercise. Muscle soreness and plasma creatine kinase accumulation following eccentric exercise were reported to be reduced [31], and this was subsequently presented as being due to an improved blood flow sustained throughout and following resistance exercise when L-carnitine was ingested [34]. However, blood flow was not monitored in these studies, and the reduced metabolic stress noted by Volek et al. [34] could be interpreted as an improved matching of substrate oxidation with demand for adenosine triphosphate (ATP). Furthermore, the evidence of less damage during exercise may be important in clinical conditions, but it could be indicative of a lesser stimulus for adaptation in athletes that may not necessarily be desirable.

It is important to remind ourselves that the overarching hypothesis from all of this work was that carnitine could somehow aid performance, particularly endurance exercise performance, through its potential impact upon metabolism, VO$_{2\text{max}}$, and blood flow/recovery. Surprisingly, many of the studies outlined above did not attempt to measure performance per se. Most studies preferred to assess markers that relate to endurance performance (e.g., VO$_{2\text{max}}$, fat metabolism, and lactate production during sustained exercise, and recovery from exercise). In recent years our understanding of factors affecting endurance performance has expanded greatly. One of the current best models for describing factors that can influence endurance exercise performance was presented by Joyner and Coyle [35]. This model (Figure 9.1) proposes that the two key factors driving improved endurance performance are the level of aerobic metabolism that can be sustained and the mechanical efficiency of movement. The first of these factors is dependent upon VO$_{2\text{max}}$, the balance between aerobic and anaerobic metabolism (lactate threshold) for maintenance of cellular ATP concentration, and the ability to perfuse active skeletal muscle. The second factor, mechanical efficiency or economy, on the other hand, is thought to be determined in large part by type I muscle fiber composition. However, efficiency is also
determined by changes in neuromuscular recruitment through self-selected preferred cadence. An optimal cadence enables an athlete to generate the most power/speed for a given oxygen cost and gain a metabolic advantage [36]. For carnitine to have an effect on endurance performance, it must act on at least one of these factors. We have already described above the potential impact that carnitine could have on cardiac muscle or peripheral oxygen extraction to improve VO\(_{2\text{max}}\). However, the whole picture also includes the potential effects of carnitine on muscle perfusion and motor unit recruitment, and carnitine as a regulator of metabolic balance through an influence on carbohydrate and fat metabolism to provide an optimal match between ATP supply and demand. As we have indicated, many of these areas have been proposed to be influenced by supplemental carnitine, but consistent evidence of beneficial effects has been lacking. Nevertheless, if carnitine could have multiple actions on these parameters, then it would not be unreasonable to suggest that it could influence endurance exercise performance.

In summary, the early work on carnitine raised more questions than answers. It is possible that many of the equivocal outcomes from these early studies reflected methodological issues such as small sample sizes; differences in exercise intensity/duration studied; adequacy of dietary intake control or reporting; differences in training status of study participants; type, dose, and duration of supplementation; and even
gender-specific responses. There were many excellent reviews published on the topic between 1990 and 2005 highlighting the proposed mechanisms for alterations in metabolism, blood flow, or markers of endurance exercise performance [37–45]. Added to the anecdotal evidence suggesting that carnitine helped the Italian football team to its 1982 World Cup win, these reviews highlighted a sufficient number of interesting positive outcomes and future directions that have guided and stimulated further research work up to the present day.

**Recent evidence**

Since 2005 over 80 original papers and reviews have been published examining the impact of carnitine administration on responses to exercise in humans. The focus in many of these studies has been on the two key areas identified previously: (1) effects of carnitine driven by increases in plasma carnitine concentration, and (2) effects due to increases in skeletal muscle total carnitine content. These two avenues have both received support over recent years, and this next section aims to summarize the more positive observations in the past 7 or 8 years, with a focus on studies investigating normal healthy humans or trained athletes.

The work of Greenhaff and colleagues in Nottingham has provided the biggest advance in our understanding of factors regulating muscle carnitine transport and muscle carnitine content. The work of this group has enabled the development of an oral carnitine ingestion strategy for manipulating muscle carnitine content. Initial work from this group by Stephens et al. [46, 47] demonstrated that hyperinsulinemia sustained for 5 hours, alongside a carnitine infusion that elevated plasma carnitine to supraphysiological concentration, resulted in an increase of muscle carnitine content by 15%. This study was the first to show that muscle carnitine content could actually be increased through exogenous administration of high-dose carnitine, and their further work [48] indicated that the presence of a high insulin concentration was required to drive the sodium-coupled transport of carnitine into skeletal muscle. The combination of high insulin in the presence of high plasma carnitine also increased the gene expression of the carnitine transporter (novel organic cation transporter 2 [OCTN2]). This suggests that a greater capacity for carnitine transport into tissues should occur if increased transporter transcription is translated into new carnitine transport proteins. Of course, these studies have also highlighted how difficult it is to increase muscle carnitine content in a practical sense; e.g., daily feeding of an oral carnitine supplement would not likely impact upon plasma carnitine or insulin in the same manner, and therefore a long period of time would be required to gradually elevate muscle carnitine content. It should also be considered that the studies by Stephens et al. [46–48] indicate that tissue sensitivity to insulin will likely impact upon
the carnitine-mediated transport capacity and suggest that regular exercisers/athletes would have greater capacity for carnitine uptake. This is something that has at least some support from an early study that revealed higher total and free carnitine content in skeletal muscle from trained individuals than from sedentary controls [49].

Stephens et al. [50] also demonstrated that the high plasma insulin and carnitine infusion protocol resulted in a reduced activity of PDC, reduced muscle lactate accumulation, and led to greater muscle glycogen storage 24 hours following the infusion. These observations were interpreted as reflecting an increase in resting fat metabolism. A decrease in glucose oxidation in favor of glucose storage to elevate muscle glycogen is a potentially positive aspect for athletes requiring fast glycogen recovery after exercise, or to support muscle glycogen loading prior to exercise. Unfortunately, no data yet exist regarding the impact of carnitine supplementation on muscle glycogen synthase activity. Stephens et al. [47] successfully went on to demonstrate that infusion of carnitine and insulin could be effectively replaced by oral carnitine plus carbohydrate ingested twice per day as a strategy to elevate carnitine retention in tissue over the long term. Indeed, based on their calculations, they suggested that in excess of 100 days of this ingestion strategy would be required to significantly elevate muscle carnitine content.

Following on from this groundbreaking work, Wall et al. [5] published the most comprehensive oral carnitine ingestion study to date. The study examined the impact of 12 and 24 weeks of oral carnitine plus carbohydrate ingestion versus carbohydrate ingestion alone, on muscle total carnitine content and substrate metabolism during low- and high-intensity exercise. In considering the effects of supplemental carnitine on skeletal muscle metabolism during low-intensity exercise they focused on whether free carnitine could be limiting to fat oxidation. For high-intensity exercise they focused on whether an enhanced acetyl group buffering role of carnitine could alter the balance of carbohydrate and fat oxidation. In addition, they examined the overall impact of carnitine supplementation for 24 weeks upon high-intensity exercise performance using a work task lasting 30 minutes. The observations from this study were the first to highlight in a single study the dual and opposite roles of supplemental carnitine upon skeletal muscle metabolism at low and high intensities of exercise. They also demonstrated for the first time an impact upon high-intensity exercise performance. During the 24-week supplementation period there was a nonsignificant decline in the mean (SEM) free carnitine content of skeletal muscle at rest in the control group [17.9 (1.8) to 15.9 (1.6) mmol (kg dry muscle)$^{-1}$] and a nonsignificant increase in free carnitine content in the carnitine-supplemented group [18.0 (1.8) to 20.7 (2.2) mmol (kg dry muscle)$^{-1}$]. The same observations were apparent for total carnitine with a slight decline of 21.4 to 19.6 mmol (kg dry muscle)$^{-1}$ in the control
group and an increase of 21.4 to 24.2 mmol (kg dry muscle)$^{-1}$ in the carnitine-supplemented group. Interestingly, the small nonsignificant increase (18%) in citrate synthase activity in the carnitine-supplemented group and 3% decline in the control group match the expectation of changes in carnitine content as reported in the early work of Cederblad et al. [51]. The absence of a statistically significant difference in muscle carnitine content between groups at rest was a little disappointing after such a long period of supplementation, but the magnitude of the difference in resting total carnitine between groups at 24 weeks is likely physiologically significant. However, there was a significant difference in free carnitine content measured following low-intensity exercise between the control and carnitine-supplemented groups at 24 weeks [11.0 (1.3) mmol (kg dry muscle)$^{-1}$ for control group vs. 19.6 (2.2) mmol (kg dry muscle)$^{-1}$ for the carnitine group]. This difference in free carnitine content suggests a greater capacity to support transport of activated fatty acids into mitochondria if free carnitine content was limiting to CPT1. Thus, an increase in fatty acid oxidation could theoretically be expected under these conditions.

The supplementation period adopted in their study was associated with small but important changes in factors that are thought to limit the capacity for fat oxidation. Indeed, following low-intensity exercise, glycogen utilization was less in the carnitine-supplemented group, suggesting a greater reliance on fat metabolism, despite a nonsignificant but higher starting muscle glycogen content. Unfortunately, whole body estimates of fat oxidation were not assessed during exercise. During the high-intensity exercise period muscle glycogen utilization was the same in control and carnitine groups; however, the muscle lactate accumulation was lower in the carnitine-supplemented group. These combined observations suggest a lower glycolytic flux during low-intensity exercise, and a greater glycolytic flux through PDC during high-intensity exercise, with their 24-week carnitine ingestion strategy. Probably most importantly, the performance of a high-intensity exercise task was improved in the carnitine-supplemented group but not in the control. The improvement amounted to an 11% increase in work output over 30 minutes at 24 weeks compared to baseline. The subtle differences in glycogen use and lactate accumulation in the prior work bouts likely contributed to an enhanced ability to undertake this high-intensity exercise task. The combination of these factors also provides an interesting basis for carnitine to benefit endurance performance following long periods of daily supplementation with a carnitine and carbohydrate mixture.

In short, the study by Wall et al. [5] provided some tempting new evidence for beneficial metabolic effects during low-intensity and high-intensity exercise that led to a change in high-intensity exercise performance. It would be interesting to posit that in an event lasting around 90 minutes, carnitine supplementation may enable an improved high-intensity
performance during the final 30 minutes. This could even provide some tangible scientific support for the anecdotal reports of carnitine improving the performance of the Italian football team in its 1982 World Cup campaign. Clearly, further work is required before this can be fully supported or applied to isolated performance tasks in a variety of sports. This work of Wall et al. [5] also requires some follow-up using whole body substrate oxidation measures to determine whether changes in whole body responses can be detected from the skeletal muscle metabolic observations.

It should be noted that one practical limitation of this supplementation protocol is the amount of carbohydrate co-ingested with L-carnitine and the recommended timing of intake (at breakfast and 4 hours later). An 80-g dose of carbohydrate constitutes a proportionally large amount of carbohydrate and around 1600 kJ of energy—equivalent to the ingestion of, for instance, 1.3 L fruit juice or 6 slices of bread or over 2 cups of cooked pasta. A twice-daily dose therefore would contribute approximately 30–40% of the total energy requirements of a smaller athlete, particularly females, and up to 70% of their total daily carbohydrate requirements. From a practical perspective, this means the carbohydrate component of this supplementation protocol would need to be ingested via a meal with a substantial contribution from carbohydrate-containing foods. In many instances, the recommended timing of these doses may impact on gastrointestinal tolerance, especially in those who train two to three times per day. Furthermore, the carbohydrate ingested in the Wall study was a glucose polymer solution, with limited information regarding foods consumed at the same time; therefore, the influence of other dietary macronutrients on the effectiveness of this supplementation strategy remains unknown.

Alongside these studies of Stephens and Wall other researchers were examining the metabolic and performance effects of shorter-term (2- to 4-week) carnitine supplementation protocols. While it is widely acknowledged that short-term supplementation with carnitine will not impact upon muscle total or free carnitine content, the potential systemic actions of carnitine can be considered by these types of study. Abramowicz and Galloway [52] observed that carnitine ingestion over 2 weeks increased the respiratory exchange ratio and subsequently the estimates of carbohydrate oxidation during moderate-intensity exercise compared with placebo in healthy active males. Broad et al. [53, 54] failed to observe differences in substrate utilization during moderate-intensity exercise following either 2 or 4 weeks of carnitine supplementation in well-trained athletes. In these studies, most of the metabolic responses were the same between trials, with the exception of a blunting of ammonia accumulation during submaximal exercise following 2 weeks of carnitine supplementation [54], suggesting a possible reduction in the metabolic stress of exercise. In addition, 20 km cycling time trial performance, following a 90-minute period of exercise, was not improved in the 4-week carnitine
ingestion study [53]. In a follow-up study [55] it was observed that the lack of metabolic effects to short-term carnitine ingestion (2 weeks) was not a result of exercise intensity. However, under conditions of high fat availability carnitine supplementation did appear to subtly influence substrate handling in metabolically active tissues, leading to a reduced circulating blood glucose concentration and reduced heart rate response during exercise at low intensities (20–60% VO₂max). These observations indicate that in well-trained individuals a short-term period of carnitine supplementation does not impact upon whole body substrate metabolism or performance, but it may have smaller systemic effects on other metabolically active tissues, such as cardiac muscle or liver, which are detectable during low-intensity exercise. From all of these studies it seems likely that large increases in skeletal muscle carnitine content would be required to induce large changes in skeletal muscle substrate oxidation, and that large changes in skeletal muscle substrate metabolism are required before this becomes measurable at the whole body level. To achieve such large effects, long-term carnitine supplementation seems required, and recent evidence suggests that this would be most likely to occur if combined with regular exercise to stimulate muscle carnitine uptake [56]. In contrast, increases in plasma carnitine concentration may influence the function of other metabolically active tissues such as cardiac muscle and liver. These possibilities require further exploration before the ergogenic benefits can be realized.

The influence of short-term carnitine supplementation on less well-trained volunteers may be of interest for a larger proportion of the exercising population. Given the differences noted in the interaction between carnitine and insulin-stimulated glucose disposal in the early study of Ferrannini et al. [57], it seems that systemic effects of carnitine on glucose homeostasis can occur. Indeed, several early reports have demonstrated that elevated plasma carnitine concentration can influence nonoxidative glucose disposal [58, 59] or can have insulin-like actions [60]. These observations, combined with the increased muscle glycogen content following short-term carnitine infusion and hyperinsulinemic clamp conditions [50], make for an interesting hypothesis that carnitine could enhance recovery from exercise by improving glucose storage rather than oxidation. For example, Galloway et al. [61] have provided some support for a beneficial effect of short-term carnitine supplementation on glucose disposal in lean active males, but did not demonstrate this effect for overweight/obese sedentary males. The difference in response between lean and overweight/obese could be due to small differences in insulin sensitivity of skeletal muscle, and therefore the capacity for skeletal muscle to act as a sink for glucose storage when challenged with a high glucose load. If this is indeed the case, then it is possible that athletes could benefit from an action of carnitine aiding the restoration of muscle glycogen when carbohydrate is ingested in the postexercise period. Other studies, largely
from Bill Kraemer’s laboratory, have also supported a role for short-term carnitine supplementation in the reduction of metabolic stress, reduction in damage/soreness, and improved recovery from exercise that may relate to improved blood flow during and following activity [62, 63]. Although these studies are promising, work is needed to verify the impact of short-term carnitine supplementation periods upon postexercise recovery and its potential subsequent impact upon adaptation to exercise and performance outcomes.

One final aspect that must be mentioned is whether vegetarian athletes have a greater requirement for supplemental carnitine given the low intake in their normal diet. Karlic et al. [64] reported that the higher carbohydrate content and low carnitine intake in the diet of vegetarians were associated with higher expression of the carnitine transporter OCTN2, and of CPT1. However, Stephens et al. [65] reported that vegetarians have a lower OCTN2 content and lower muscle total carnitine content. A number of factors such as physical activity status need to be controlled for in studies of this nature, and more research is needed to further explore and understand these observations.

Conclusions/practical recommendations

A large body of literature on carnitine has contributed to significant progress in recent years, and we now understand a great deal more about how to increase muscle carnitine content and the potential systemic effects of elevated plasma carnitine. However, it should be apparent from the lack of firm conclusions in reviewing this work that we are still in the early stages of our understanding of carnitine as a potential ergogenic aid for sport performance. Indeed, we have yet to get to the stage where we can begin to undertake a variety of performance-related studies. As such, the ruling in June 2011 by the European Food Safety Authority that carnitine does not increase endurance capacity, and does not aid faster recovery from exercise, seems fair at this stage. In 5–10 years time the opinion may be different if the promising initial observations can be supported and refined.

It may be that new ideas and avenues must be explored, such as developing a greater understanding of the importance of subcellular locations of carnitine (cytosolic vs. intramitochondrial). For example, there is evidence of peroxisomal and nuclear carnitine acetyl transferase (CAT) activity in humans [66, 67] that could generate an increase in cytosolic free carnitine. A rise in free carnitine in the cytosol can reduce CPT1 sensitivity to malonyl-CoA [66], an inhibitor of CPT function and consequently of fatty acid oxidation. It has long been known that CPT1 sensitivity to malonyl-CoA is reduced during exercise, but the possibility of cytosolic free carnitine being involved would be an interesting mechanistic proposal. There
is also evidence [68] that deletion of muscle-specific CAT compromises substrate handling (glucose tolerance and metabolic flexibility) in mice.

Even if new avenues of research are not explored, it seems that there are several practical questions arising from the present work that must be addressed before carnitine can be fully accepted as an ergogenic aid: (1) Can we find ways of shortening the period of supplementation required to induce changes in muscle carnitine content? (2) Could a smaller amount of carbohydrate co-ingested with carnitine result in similar changes in muscle carnitine content? (3) Can we evaluate the systemic changes in carnitine concentration that are necessary to consistently demonstrate an impact upon cardiac function/liver function or responses related to recovery from exercise? (4) Can carnitine supplementation consistently impact upon the parameters that are known to be key drivers for improved endurance exercise performance or recovery?

In translating this to real-life exercise and sport participation, the potential ergogenic benefit of carnitine supplementation (within the context of the current supplementation protocol) is most likely going to be confined to well-trained athletes who already have well-adapted metabolic systems, whose frequency of training may result in reduced muscle carnitine stores, and who already have consistently repeatable performances. The evidence to date suggests there would be limited benefits to short-duration high-intensity exercise bouts (e.g., <2 minutes), such as many athletics, swimming and track cycling events, and skill or aesthetic sports such as gymnastics, diving, shooting, and archery. The potential for improved glycolytic flux may benefit events of 2–7 minutes of high intensity where lactate accumulation can limit performance (such as rowing, 800–1500 m running, 400–1500 m swim events, and 500–1000 m sprint canoe/kayak events), and potentially some combat sports (such as boxing). The potential to improve both fatty acid utilization and glycolytic flux may benefit events of moderate duration but relatively high intensity, such as cycling time trials and 5–10 km running, as well as sports requiring intermittent high-intensity work (e.g., most team and racquet sports). There may also be benefits for endurance events where performance may be limited by glycogen stores, and where opportunities for carbohydrate intake during the event may be limited or involve frequent changes in pace (such as Olympic distance triathlon and marathon). Whether the degree of change in muscle carnitine status and its glycogen sparing effect would be clinically relevant enough to benefit prolonged endurance performance may be questionable. This would be particularly true when the majority of work is undertaken at steady state and there is ample opportunity to consume carbohydrate during the event (such as road cycling stage races or Ironman triathlon). The biggest impact of carnitine supplementation may in fact be on supporting effective and consistent training and enabling the athlete to fully stress the system during training, rather than
purely the performance of a single competitive event; however, this will be difficult to assess considering the long duration of supplementation currently recommended.

References


Summary remarks and future directions

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Skeletal muscle is responsible for a significant proportion of whole body oxygen consumption, reflecting its need to combust nutrient substrates in order to provide sufficient adenosine triphosphate (ATP) to sustain muscular contractions. Carnitine occupies a unique position in skeletal muscle energy metabolism, being involved in both fat and carbohydrate metabolism. As such, the study of carnitine metabolism in skeletal muscle offers insight into the limitations of fuel metabolism responsible for detriments in muscle function in the context of both chronic illness and acute fatigue. In the two decades since the last text on carnitine was published (Current Concepts in Carnitine Research, CRC Press, 1991), significant strides have been made in terms of understanding key aspects of muscle carnitine homeostasis and function. As such, an up-to-date text on the role of carnitine in human metabolism and, in particular, skeletal muscle metabolism, was warranted.

In editing this text, we set out to contact colleagues who are active researchers in the field of carnitine metabolism and human nutrition to act as contributing authors, and we are happy to say that we have been successful in this endeavor. As such, the current text distills the latest concepts from scientists at the forefront of carnitine-related research. We are extremely grateful to all of our colleagues for their excellent contributions, without which this text would not have been possible.

In the opening section of the text, Dr. Borum (Chapter 1) provides an overview of carnitine homeostasis in humans. Thereafter, the book follows a (skeletal) muscle-centric theme, where Drs. Altamimi and Lopaschuk (Chapter 2) describe the role of carnitine on the metabolic regulation of fuel metabolism in heart and skeletal muscle. Section 2 of the text focuses exclusively on skeletal muscle carnitine metabolism. Following an introduction to the role of carnitine in skeletal muscle fuel metabolism (Chapter 3), Dr. Stephens discusses skeletal muscle carnitine transport, offering insight as to why achieving skeletal muscle carnitine loading in humans
is not a trivial matter. Thereafter, the metabolic consequences of altering muscle carnitine levels are discussed in detail (Chapter 5).

The final section of the text focuses on the potential application of carnitine supplementation in specific clinical populations and the role of carnitine as an ergogenic aid for athletes. In Chapter 8, Dr. Andreozzi provides us with evidence for a beneficial role of carnitine supplementation in patients with intermittent claudication associated with peripheral arterial disease. In Chapter 6, Dr. Brass discusses the impact of dialysis in patients with end-stage renal failure on carnitine homeostasis, and whether carnitine treatment is warranted in this patient population. In Chapter 7, Drs. Wicks, Noland, and Mynatt offer a comprehensive mechanistic overview of skeletal muscle insulin resistance, while reviewing perhaps one of the more active fields of carnitine research: the role of carnitine or carnitine shuttle systems in the metabolic abnormalities associated with obesity and the metabolic syndrome. Finally, Drs. Galloway and Broad offer a fresh perspective on an old question—whether carnitine can act as an ergogenic aid for athletic performance (Chapter 9).

In reviewing the aforementioned chapters of this text, several points are apparent. In the first instance, from a basic muscle biochemistry perspective, carnitine’s dual role in energy metabolism positions it as a unique regulator of the integration of muscle fat and carbohydrate metabolism in humans. Subsequently, (1) acute or chronic alterations in muscle fat and carbohydrate metabolism may be mediated by a limitation in carnitine availability or the activity of the carnitine palmitoyl transferase (CPT) and carnitine acetyl transferase (CAT) enzymes, and (2) these alterations in muscle fat and carbohydrate metabolism may be responsive to muscle carnitine loading/depletion. From a nutritional perspective, with regards to peripheral arterial disease (Chapter 8) and end-stage renal failure (Chapter 6), there is clear academic rationale for the application of carnitine as a nutritional therapy. However, it would appear that the jury is still out as to whether carnitine supplementation can, in fact, impact muscle carnitine levels and subsequently improve clinical outcomes in these patient populations. As such, these situations remain active areas for current research. Similarly, while mechanistic insight has grown substantially regarding the role of carnitine in the dysregulation of fuel metabolism in obesity and insulin resistance, data truly assessing the benefits of long-term supplementation in humans remain to be gathered (Chapter 7). Indeed, a common theme with regards to the application of carnitine supplementation in patient groups with metabolic myopathies that may be responsive to carnitine therapy is the paucity of large human clinical trials. Performing these large cohort long-term human studies should be a research priority moving forward if we are to translate the considerable body of mechanistic science regarding carnitine metabolism into tangible clinical benefits at a population level.
Concerning the potential of carnitine to preferentially alter muscle energetics and therefore act as an ergogenic aid in the sports arena, evidence is currently lacking, owing largely to the difficulties associated with augmenting muscle carnitine availability in healthy humans. The relatively recent demonstration that specific long-term feeding protocols can augment muscle carnitine stores will hopefully renew interest in this avenue of research, and the potential for carnitine administration to augment performance across a range of athletic endeavors can be investigated. In closing, the last 20 years of carnitine research have been fruitful. In distilling some of the key areas of muscle carnitine research over this time period in one text, we hope to have produced a reference for students, researchers, and clinicians with an interest in muscle metabolism and carnitine. Moreover, a clear theme throughout the current text is that while significant advances in our knowledge have been made, many key questions remain unanswered. It is our hope that in the next 20 years some of these key questions will be comprehensively addressed so that the role of carnitine in skeletal muscle metabolism in health and disease can be more fully understood, and where appropriate, carnitine therapy can be applied as an evidence-based approach.
Carnitine Metabolism and Human Nutrition

*Carnitine Metabolism and Human Nutrition* offers a contemporary and in-depth look at the biological effects of carnitine metabolism and its application to clinical and sports nutrition based on decades of robust scientific enquiry. It gathers and distills key results of the last 20 years of carnitine research to provide an invaluable reference tool for students, researchers, and clinicians.

This book addresses the importance of carnitine in skeletal muscle fuel metabolism, the complexities and importance of muscle carnitine transport, and the metabolic insight that has been gained from experiments manipulating muscle carnitine stores.

The authors cover the potential application of carnitine supplementation in specific clinical populations and the role of carnitine as an ergogenic aid for athletes. They also provide a comprehensive mechanistic overview of skeletal muscle insulin resistance, including the role of carnitine shuttle systems in the metabolic abnormalities associated with obesity and the metabolic syndrome.

*Carnitine Metabolism and Human Nutrition* provides you with a comprehensive and up-to-date look at the properties and underlying metabolic biochemistry of carnitine. The book includes contributions from leading international scientists, each a pioneer in their chosen study of carnitine metabolism or its application to human nutrition.