ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

L-carnitine of red meat consumption contributes less in heart diseases progression as compared to low expressed PPARα leading to activation of mTOR1C

Umar Bacha*[1], Muhammad Nasir[1], Muhammad Asif Ali[1],Naveed Akbar[1], Aftab Ahmad Anjum[2], Javed Muhammad[2], Tanveer Hussain[3], Abdul Wajid[3], Tayyab Shahzad[1], Anjum Rashid[4]

1. Department of Food Science & Human Nutrition, University of Veterinary & Animal Sciences, Lahore, Pakistan.
2.Department of Microbiology University of Veterinary & Animal Sciences, Lahore, Pakistan.
3. Institute of Biochemistry and Biotechnology, University of Veterinary & Animal Sciences, Lahore, Pakistan.
4. Department of Dairy Technology, University of Veterinary & Animal Sciences, Lahore, Pakistan.

Manuscript Info *Abstract*

Manuscript History:

Received: 21 August 2013
Final Accepted: 31 August 2013
Published Online: September 2013

Key words:
L-Carnitine, Microbiota,
PPAR-α, mTOR1C,
CVD, Meat processing.

Carnitine, choline and polyamines are conditionally essential compounds due to its specific role in the body particularly, lipid transportation across plasma membrane. The compound decreases triglyceride, cholesterol and VLDL. Considering these findings, it appears therefore, that L-carnitine supplementation might help in reduction of cardiovascular diseases and diabetes. Because, carnitine level ablated in patients with type 2 Diabetes Mellitus. However, regarding processed meat intake, posed association with coronary heart diseases and diabetes mellitus which is attributed to L-carnitine present in meat. The present review evaluates findings of the different studies carried out so far and resolves the confliction about L-carnitine of red meat origin and risk of cardiovascular (CVD) diseases. Red meat could provide other substances to the gut microbes which then activates mTOR1C and suppresses PPAR-α. This effect may be responsible for risk of CVD. It is therefore, imperative to carry out cooking practices on carnitine level reduction and then processed meat evaluation in heat patients. This type of study may unravel the mystery about the actual culprit behind CVD indices.

Introduction

1. Carnitine and Heart Diseases

Apart from some amino acids and vitamins; carnitine, choline and polyamines are conditionally essential compounds (Gallego et al., 2006) due to its specific role in the body. Carnitine plays pivotal role in lipid transportation (Vidal-Casariego et al., 2013). Addition of L-carnitineto dietsdecreases ($p < 0.05$) triglyceride, cholesterol and VLDL (Rezaei et al., 2007) and improves body-weight gain (Rabie and Szilágyi, 1998) in broilers. A significant increase in body weight gain and reduction in abdominal fat is also reported (Rabie et al., 1997) in broilers. Considering these findings, it appears therefore, that L-carnitine supplementation might help in reduction of cardiovascular diseases and diabetes. A systematic review and a meta-analysis reported that red meat intake is not associated with coronary heart diseases (n=4 studies) and diabetes mellitus (n=5). However, carnitine level ablated in patients with type 2 Diabetes Mellitus (T2DM). A systematic review on (n= 284) patients to confirm beneficial effects of this compound on indices of diabetes and obesityhad reveals low fasting plasma glucose, total cholesterol and LDL level (Vidal-Casariego et al., 2013).On the other hand, processed meat intake posed association withcoronary heart diseases and diabetes mellitus (Micha et al.,2010). Another study has reported a significant reduction (27%) inall-cause mortality and angina (40%) after L-carnitine supplementation(DiNicolantonio et al., 2013).

2. Carnitine and TMA/TMAO production

However, there are conflicting results regarding L-carnitine of red meat origin and risk of cardiovascular (CVD) diseases.Trimethyl-amine (TMA) is generated from dietary L-carnitine (TMA), choline and phosphatidylcholineby gut microbiota which is further churn down into a pro-atherogenic substance, tri-methylamine-N-oxide (TMAO) is reported to accelerate atherosclerosisin mice. Longer L-carnitine supplementationin mice resulted in altered cecal microbiota with high level of TMA, TMAOand atherosclerosis. The effects were found vanished when intestinal microbiota growth is suppressed with the use of antibiotics. This indicates that gut microbiota have definite role in generation of pro-atherogenic substances from red meat (Koeth et al., 2013).

Omnivorous human is therefore, at risk for TMAO production as compared to vegans or vegetarians and high level of TMAO is related toparticular bacterial taxa in the human intestine. Not only L-carnitine but also phosphatidylcholine give rise to TMAO production via gut microbiota and therefore, serves as possible risk of CVD adverse events (joseph, 20 13).Similarly, betaine is predicted to implicate in risk of CVD in clinical cohort(Wang et al., 2011).Choline, carnitine etc., are the potential precursors of TMA and TMAO (Zhang et al., 1999) whereas betaine, creatinine and lecithin although, generate these compounds but less efficiently. However, total choline production in the body arises from glycerol-phosphocholine, phosphocholine, phosphatidylcholine and sphingo-myelin. Similarly, choline may originate from food sources as well (Table. 1). Phosphatidylcholine of food stuff through microbial metabolism in the gut generate choline and then subsequently to TMAO production which then contribute to cardiovascular events in humans subjects (Tang et al., 2013).

Plant with capability of producing enzymes amino-aldehyde dehydrogenases (AMADHs) carry out polyamine catabolism and carnitine biosynthesis. Tomato, peas and maize also synthesizes isoforms of AMADHs and are linked to carnitine production (Kopecny et al., 2013). However, these plant sources are also rich in dietary fibers and it is possible that it promotes growth of health friendly bacteria. Hence, contribution of carnitine production in the plant sources may not act as threat. Because, whole grain cereal (WG) diet consumption lower urea, carnitine and acylcarnitines in the urine as compared to refine grain diet in the subjects(Ross et al., 2013). However, further research might be needed on WG, refined grain and red meat intervention for a clearer difference of carnitine level in either blood plasma or urine. Enzymes flavin mono-oxygenase (FMO1 and FMO3) is shown to oxidize TMA to TMAO. Moreover, FMO3 is a potential candidate (10-fold higher) for oxidation as compared to FMO1 in mice and FMO3 overexpression resulted in significant elevation of plasma TMAO levels and vice versa. Thus, therapeutically it is possible and necessary to search out antagonist of this enzyme because FMO3 level is significantly correlated with TMAO production (Bennett, 2013).

3. PPAR-α genes expression and carnitine

Peroxisome proliferator activated receptor α (PPAR-α)actively participate in lipid metabolism which could impart clinically beneficial effects on body because, PPAR-α genes over-expression are related with decreasedcoronary vascular events and mortality. Animals and in vitro studies have revealed anti-inflammatory and anti-atherosclerotic effects of PPAR-α agonists, particularly, in the artery walls(Francis et al., 2003). L-carnitine upon protein degradation is synthesized (Fig. 1) in liver,muscle and kidneys. The rate of synthesis is however, affected by PPAR-α genes expression level (Koch et al., 2008).Similarly, mammalian target of rapamycin complex 1 (mTORC1) kinase actively participate in cell growth which depend on nutrients, energy and growth factors availability. However, mTORC1 inhibition through certain inhibitors activates PPARα. The mTORC1 starts signaling when a nuclear receptor co-repressor 1(NCoR1), a co-repressor of PPARα, is suppressed thereby, silencing to "ketogenesis in cells"(Sengupta et al., 2010).Carnitine palmitoyl-transferase (CTP1C) overexpressed level lower or inhibits mTOR1C signaling. On the other hand, a potent mTORC1 activator is leucine amino acid (Schriever et al., 2012). Leucine allosterically attaches to glutamate dehydrogenase enzyme and churn down glutamate to alpha keto-glutarate (αKG) which in turn triggered mTORC1 through prolyl hydroxylase enzyme activation. Hence, we can say that mTOR1C sense leucine this way. Noteworthy, glutamate dehydrogenase activation via leucine also led to secretion of insulin from pancreatic β cells. Therefore, in type II diabetes, dietary intervention having leucine and glutamine is needed to be explored. Gut microbiota is therefore needed to be elucidated because a huge number of gut microbiota express glutamate dehydrogenases and glutamine synthesis (Kolmeder et al., 2012) which may helpful in insulin secretion however, in diabetic heart patient could increase risk of CVD because of PPARα lower expression.Bjørndal et al. (2013) reported a significant increase in leucine level in response to FO

(fish oil), TTA (tetr-adecyl-thio-acetic acid) and combination of both FO and TTA diet. Logically, based on above facts, it appears that after feedingor nutrient intake,mTORC1 interacts with NCoR1, through phosphorylation of S6K2, (Mottis et al., 2013) leading to a decrease activity of PPARα. If this process really happening then carnitine production should be lower down because depressed PPARα would depress carnitine synthesis in the body. Therefore, apparently, L-carnitine contributes less in heart diseases; rather, it is PPARα low expression which results from protein degradation (e.g leucine liberation) that activatesmTOR1C leading NCoR1 activation.

PPARαligands suppressed osteo-pontin (OPN), a pro-inflammatory cytokine implicated in the chemo-attraction of monocytes and the development of atherosclerosis, promoter activity(Nakamachi et al., 2007).High fructose feeding cause's atremendous increase in hypertriglyceridemia and decreases insulin sensitivity via suppression of hepatic PPARα. However, the process is reversed with treatment lipoxygenase/cyclooxygenase compounds as anti-inflammatory compounds (Kelley and Azhar, 2005).Recently, PPARα target genes and PPARαcaptivators (ACOX1, CPT1A, ACADVL, ACSL1, PPARA, PPARGC1A, LPIN1) expression were tested in the presences of PPARα ligand Wy-14643 (WY) and A16:0, as a representative of long chain fatty acids. The results indicated about 500% increase in CPT1A gene expression (Thering et al., 2009).Akt is an effector protein kinase and upon activation (phosphorylation) promotes glycogen synthesis in the tissues (Fritsche et al., 2008; Saltiel andKahn, 2001). However, lipids insult i.e. palmitate prevents Akt (i.e. Akt, S6K1) activation (Caroline et al., 2012), as a result, glycogen synthesis is inhibited. In a nutshell, carnitine in meat products may not act as CVD risk rather, these deactivators of PPARs and agonist of mTOR1C.

Table 1: Choline contents of different food stuff

Food stuff	Quantity of Choline (mg/100g)
Beef liver	418
Chicken liver	290
Eggs	251
Wheat germ	152
Wheat bran	1339*
Wheat germ	1241
Spinach	645
Pretzels	237
Shrimp	218
Wheat bread	201
Bacon	125
Dried soybeans	116
Pork	103

*= Highest quantity of choline; Data taken from Zeisel et al., 2003.

Table 2: Bacterial strains producing Carnitine

Bacteria type	Substrate	Reference
***Escherichia coli* and *Proteus sp*.**	Crotonobetaine or D-carnitine	Bernal et al., 2007
Saccharomyces cerevisiae, Penicillium, Rhizopus, Mucor, Actinomucor, Neurospora, Aspergillus, Achromobacter, Pseudomonas, Nocardiacrassa	γ-butyrobetaine	Bernal et al., 2007
AcinetobacterCalcoaceticus*	D-Carnitine	Naidu et al., 2000
Fusariumoxysporum	D,L-Octanoylcarnitine	Aragozzini et al., 1986
***Escherichia coli* O44 K74**	D(+)-carnitine	Castellar et al., 1998
***Escherichia coli* O44 K74**	Fumarate	Castellar et al., 1998
Escherichia coli	Trimethylamonium compounds	Canovas et al., 2003

*=Microorganism of Choice for conversion of D-Carnitine to L-Carnitine

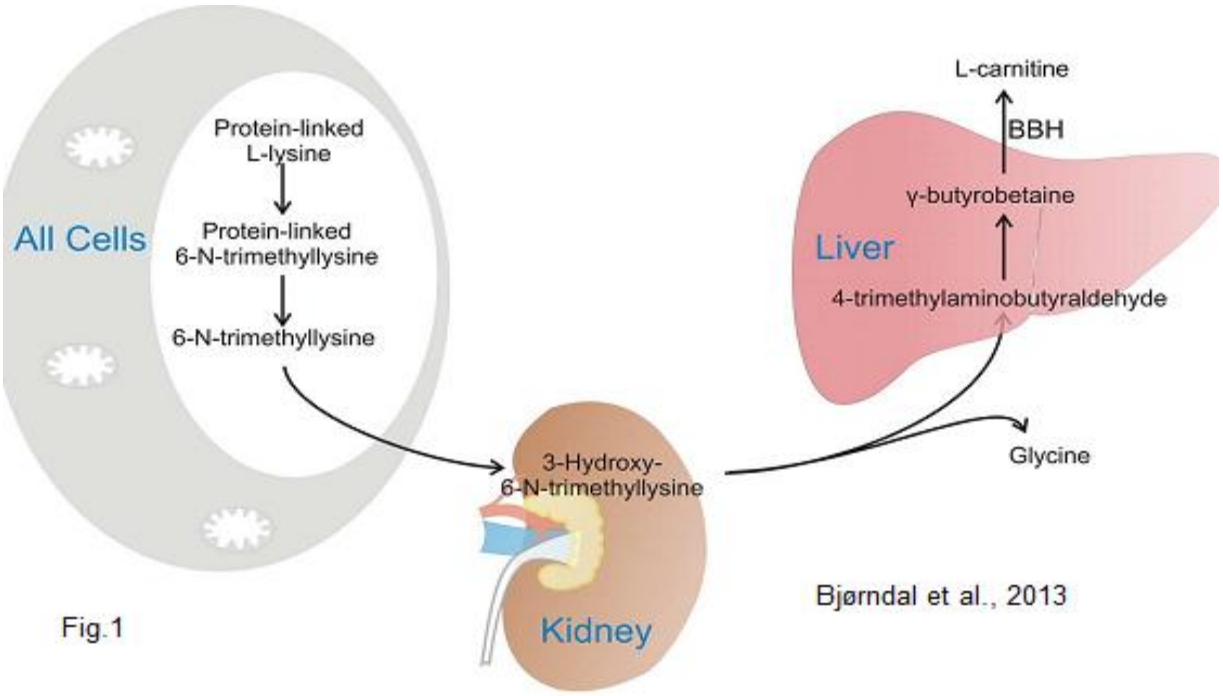

Fig.1

References

Abou-Seif, R.A., Ebrahim, M.S.M. and Radwan, M.E.I. (2009): The use of tomato seeds by-product meal supplemented with L. carnitine as non-conventional ingredients in practical diets for monosex Nile tilapia, Oreochromisniloticus. Mansoura University Journal of Agricultural Sciences, 34 (6): 1110-0346.

Aragozzini, F., Manzoni, M., Cavazzoni, V. and Craveri, R. (1986): D,L-carnitine resolution by Fusariumoxysporum. Biotechnol.Lett. 8: 95-98.

Bennett, B.J.,Thomas, Q. de AguiarVallim.,Wang, Z., Shih, D.M., Meng, Y., Gregory, J., Allayee, H., Lee, R.,Graham, M., Crooke, R., Edwards, P.A., Hazen, S.L. and Lusis, A.J. (2013): Trimethylamine-N-Oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation, Cellmetabolisim, 17 (1): 49–6.

Bernal, V., Sevilla, A., Cánovas, M. and Iborra, J.L. (2007): Production of L-carnitine by secondary metabolism of bacteria. Rev. Microbial Cell Factories, 6:31.

Bjørndal, B., Brattelid, T., Strand, E., Vigerust, N.F., Svingen, G.F.T., Svardal, A., Nygard, O. and Berge, R.K. (2013): Fish Oil and the Pan-PPAR Agonist Tetradecylthioacetic Acid Affect the Amino Acid and Carnitine Metabolism in Rats. PLoS ONE 8(6): e66926.

Canovas, M., Bernal, V., Torroglosa, T., Ramirez, J.L., Iborra, J.L. (2003): Link between primary and secondary metabolism in the biotransformation of trimethylammonium compounds by escherichia coli. Biotechnology and Bioengineering, 84:6.

Caroline, S.A. and Laybutt, D.R. (2012): Lipid-Induced Endoplasmic Reticulum Stress in Liver Cells Results in Two Distinct Outcomes: Adaptation with Enhanced Insulin Signaling or Insulin Resistance. Endocrinology, 153 (5): 2164–2177.

Castellar, M.R., Canovas, M., Kleber, H.P. and Iborra, J.L. (1998): Biotransformation of D (+)–carnitine into L(−)–carnitine by resting cells of Escherichia coli O44 K74. Journal of Applied Microbiology, 85: 883–890.

DiNicolantonio, J.J., Lavie, C.J., Fares, H., Menezes, A.R. and Okeefe, J.H. (2013): L-Carnitine in the Secondary Prevention of Cardiovascular Disease: Systematic Review and Meta-analysis. Mayo Clinic Proceedings, 88 (6): 544–551.

Francis, G.A., Annicotte, J.S. and Auwerx, J. (2003): PPAR-α effect on the heart and other vascular tissues. AJP – Heart, 285(1): H1-H9.

Fritsche, L., Weigert, C., Haring, H.U. and Lehmann, R. (2008): How insulin receptor substrate proteins regulate the metabolic capacity of the liver–

implications for health and disease. CurrMedChem.15:1316–1329.

Gallego, L.F., SáezLara, M.J., Bailón, R.S. and Hernández, A.G. (2006): Nitrogenous compounds of interest in clinical nutrition. Nutr Hosp. 21 (S2:14-27): 15-29.

Joseph, L. (2013): Gut Microbiota, the Genome, and Diet in atherogenesis. New England Journal of Medicine 368:17, 1647-1649.

Kelley, G.L.andAzhar, S. (2005): Reversal of High dietary fructose-induced PPARα suppression by oral administration of lipoxygenase/cyclooxygenase inhibitors. Nutrition & Metabolism, 2:18.

Koch, A., Konig, B., Stangl, G.I. and Eder, K. (2008): PPAR alpha mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. ExpBiol Med (Maywood) 233: 356–365.

Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T, Britt, E.B., Fu, X., Wu, Y., Li, L., Smith, J.D., DiDonato, J.A., Chen, J., Li, H., Wu, G.D., Lewis, J.D., Warier, M., Brown, J.M., Krauss, R.M.,Tang, W.H., Bushman, F.D., Lusis, A.J. and Hazen, S.L. (2013): Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nature Medicine, 19: 576–585.

Kolmeder, C.A., De Been, M., Nikkilä, J., Ritamo, I., Mättö, J., Valmu, L., Salojärvi, J., Palva, A., Salonen, A. and DeVos, W.M.(2012): Comparative Metaproteomics and Diversity Analysis of Human Intestinal Microbiota Testifies for Its Temporal Stability and Expression of Core Functions. PLoSONE. 7(1): e29913.

Kopecny, D., Koncitikova, R., Tylichova, M., Vigouroux, A., Moskalíkova, H., Soural, M., Sebela, S. and Moréra, S. (2013): Plant ALDH10 Family identifying critical residues FOR substrate specificity and trapping a thiohemiacetal intermediate. JBC. 288: 9491-9507.

Micha, R., Wallace, S.K. and Mozaffarian, D. (2010): Red and Processed Meat Consumption and Risk of Incident Coronary Heart Disease, Stroke, and Diabetes Mellitus: A Systematic Review and Meta-Analysis, Circulation, 121: 2271-2283.

Mottis, A., Mouchiroud, L. and Auwerx, J. (2013): Emerging roles of the corepressors NCoR1 and SMRT in homeostasis. Rev, Genes & Development, 27:819–835.

Naidu, G.S.N., Lee, I.Y., Lee. E.G., Kang, G.H. and Park, Y.H. (2000): Microbial and enzymatic production of L-Carnitine. Bioprocess Engineering 23: 627-635.

Nakamachi, T., Nomiyama, T., Gizard, F., Heywood, E.B., Jones, K.L., Zhao, Y., Fuentes, L., Takebayashi, K., Aso, Y., Staels, B., Inukai, T. andBruemmer, D. (2007): PPARalpha agonists suppress osteopontin expression in macrophages and decrease plasma levels in patients with type 2 diabetes. Diabetes, 56 (6):1662-70.

Rabie, M.H. andSzilágyi, M. (1998): Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. Br J Nutr. 80 (4):391-400.

Rabie, M.H., Szilágyi, M.andGippert, T. (1997): Effects of dietary L-carnitine supplementation and protein level on performance and degree of meatness and fatness of broilers. ActaBiol Hung. 1997;48(2):221-39.

Rezaei, M., Attar, A., Ghodratnama, A.and Kermanshahi, H. (2007): Study the effects of different levels of fat and L-carnitine on performance, carcass characteristics and serum composition of broiler chicks. Pak J Biol Sci. 10(12):1970-6.

Ross, A.B., Pere-Trépat, E., Montoliu, I., Martin, F.P., Collino, S., Moco, S., Godin, J.P., Cléroux, M., Guy, P.A., Breton, I., Bibiloni, R., Thorimbert, A., Tavazzi, I., Tornier, L., Bebuis, A., Bruce, S.J., Beaumont, M., Fay, L.B.and Kochhar, S. (2013): A whole-grain-rich diet reduces urinary excretion of markers of protein catabolism and gut microbiota metabolism in healthy men after one week. J Nutr. 143(6):766-73.

Sahin, N., Orhan, C., Tuzcu, M., Sahin, K.andKucuk, O. (2008): The effects of tomato powder supplementation on performance and lipid peroxidation in quail. Poult Sci. 87 (2): 276-83.

Saltiel, A.R. and Kahn, C.R. (2001): Insulin signalling and the regulation of glucose and lipid metabolism. Nature, 414:799–806.

Schriever, S.C., Deutsch, M.J., Adamski, J., Roscher, A.A.andEnsenauer, R. (2012): Cellular signaling of amino acids towards mTORC1 activation in impaired

human leucine catabolism. J Nutr Biochem.2012, (2012).

Sengupta, S., Peterson, T.R., Laplante, M., Oh, S. and Sabatini, D.M. (2010): mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature, 468 (7327): 1100-4.

Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y and Hazen SL (2013). Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk. N Engl J Med. 368:17.

Thering, B.J., Bionaz, M. and Loor, J.J. (2009): Long-chain fatty acid effects on peroxisome proliferator-activated receptor-alpha-regulated genes in Madin-Darby bovine kidney cells: optimization of culture conditions using palmitate. J Dairy Sci. 92(5): 2027-37.

Vidal-Casariego, A., Burgos-Peláez, R., Martínez-Faedo, C., Calvo-Gracia, F., Valero-Zanuy, M.Á., Luengo-Pérez, L.M. and Cuerda-Compés, C. (2013): Metabolic effects of L-carnitine on type 2 diabetes mellitus: systematic review and meta-analysis. ExpClinEndocrinol Diabetes, 121 (4):234-8.

Wang, Z., Klipfell, E., Bennett, B.J., Koeth, R., Levison, B.S., Dugar, B., Feldstein, A.E., Britt, E.B., Fu, X., Chung, Y.M., Wu, Y., Schauer, P., Smith, J.D., Allayee, H., Tang, W.H., DiDonato, J.A., Lusis, A.J. and Hazen, S.L. (2011): Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature, 472 (7341): 57-63.

Zeisel., S.H., Mar, M.H., Howe, J.C. andHolden, J.M. (2003): Concentrations of choline-containing compounds and betaine in common foods. *J. Nutr*. 133, 1302–1307.

Zhang., A.Q., Mitchell, S.C. and Smith, R.L. (1999): Dietary precursors of trimethylamine in man: a pilot study. Food Chem. Toxicol. 37, 515–520.