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Rethinking the Regulation of L-Carnitine Transport in Skeletal Muscle Cells. Focus on "the Role of AMPK"

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Running Title: The Role of AMPK in the Regulation of L-Carnitine Transport

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Carnitine is a critical cofactor in the metabolism of lipids and therefore in the production of cellular energy. L-Carnitine, the active form, plays an important role in oxidizing fatty acids, transporting long chain fatty acids across mitochondrial membrane and modulating intracellular coenzyme A homeostasis (3). L-Carnitine uptake into cells is mediated primarily by the organic cation/carnitine transporters (OCTN), a subclass of the solute carrier 22 transporter family. Patients bearing mutations in OCTN2 gene exhibit severe symptoms because of the resulting cardiomyopathy, progressive skeletal weakness, nonketotic hypoglycemia, and hyperammonemia (10). Currently the information on the regulation of L-Carnitine transport into skeletal muscle cells is sparse. AMP-activated protein kinase (AMPK), a cellular energy sensor, usually acts to inhibit energy-utilizing pathways (e.g., fatty acid and protein synthesis) while boosting energy-generating pathways (e.g., glucose uptake and fatty acid oxidation) (2). Like AMPK, insulin has been shown to promote the uptake of energy-generating molecules such as glucose and fatty acids (6, 9). However, the information on whether there is a direct linkage between AMPK, insulin and L-Carnitine transport in muscle cells is currently missing.

In this issue of *American Journal of Physiology-Cell Physiology*, Shaw and colleagues (8) tested the hypothesis that both AMPK and insulin enhance L-Carnitine transport into skeletal muscle cells through the members of the OCTN family. Using mouse myoblast cell line C2C12 as a model system, the authors first confirmed the existence of the OCTN family of the transporters OCTN1/2/3 at both gene and protein levels. Subsequently, the authors examined L-Carnitine uptake into the cells in the presence of insulin or various AMPK activators. As expected, there is a modest increase for L-Carnitine uptake when cells were treated with insulin at a relatively high concentration. However, as contrary to their hypothesis, all AMPK activators used significantly inhibited carnitine uptake (Fig. 1). The inhibition by caffeine, one of the activators used, was partially reversed by an AMPK inhibitor Compound C, indicating the requirement of AMPK in this process. The demonstration of the negative relationship between AMPK activation and L-

Carnitine transport is novel, because it challenges the common notion that AMPK, as a cellular energy guardian, would promote the uptake of L-Carnitine, a critical player in fatty acid metabolism and cellular energy creation. This study provides fresh insights into the pharmacological treatment of L-Carnitine deficiency for the improvement of L-Carnitine accumulation in skeletal muscle to increase metabolism.

Indeed, the opposite linkage between AMPK activation and L-Carnitine transport, discovered by Shaw and colleagues (8), is intriguing, and is the first step into a fascinating area of investigation. Many questions remain to be answered: (a) although the authors detected the expression of OCTN1/2/3 in C2C12 cells, it would be imperative to address whether AMPK indeed regulates L-Carnitine uptake through these transporters. If so, which specific OCTN isoform is involved? It should be noted that transporters other than OCTN1/2/3 could also transport L-Carnitine, including OCT6 and amino acid transport system $B^{o,+}$ ($ATB^{o,+}$) (10). (b) once the specific carnitine transporter is identified, and the involvement of AMPK is confirmed, it would be interesting to explore the mechanism underlying the regulation by AMPK. AMPK may affect transporter activity by altering the binding affinity of the transporter to its substrates, by altering the expression, membrane trafficking, and stability of the transporter, or by altering the interaction of the transporter with its interacting partners (7). (c) The AMPK activators used in this study (except caffeine, which exerts it role, at least in part, through AMPK) may function to bypass AMPK and act directly on the transporters as competitive or non-competitive inhibitors, leading to the inhibition of L-Carnitine transport. Thus, it would be important to assess this possibility. (d) although the inhibitory effect of AMPK on L-Carnitine transport in C2C12 cells contrasted with what was expected of a stimulatory effect, the opposite regulation by AMPK on the membrane transporter has been observed in other tissues, contingent on the timing and chronicity of AMPK activation. For example, acute activation of AMPK causes a downregulation of Na^+-dependent glucose transporter SGLT1 in gut (5), whereas chronic activation of AMPK in

transgenic mice bearing a gain-of-function mutation in AMPK causes an enhanced SGLT1 activity in heart (1). Therefore, the behavior of AMPK in C2C12 cells may bear such analogy. (e) AMPK is a heterotrimeric kinase consisting of one catalytic α-subunit and two regulatory β- and γ-subunits. Although it has been acknowledged that each subunit exists in multiple isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) with various expression level and tissue distribution, the exact function of these isoforms remains unknown. For example, human heart is reported to have a large amount of α1-AMPK activity, whereas α2-AMPK accounts a majority role in mouse heart (4). Since the current study is using mouse myoblast cell line, it is possible that the difference in the composition of AMPK isoforms between human and rodent could cause the unexpected effect on L-Carnitine uptake. (f) finally, it should also be kept in mind that, depending on the cell type, protein-protein interactions, signaling pathways, and the level of expression of the individual transporter can be quite different. Therefore, it is crucial to confirm and extend the current findings from C2C12 cells (8) in another cell line, or in primary cells, or even in vivo so that the regulation of L-carnitine transport in muscle cells can be thoroughly understood.

Taken together, to our knowledge, Shaw and colleagues have pioneered in the field of AMPK regulation of L-Carnitine transport in skeletal muscle cells. Further exploration in this area could lead to thrilling implication in our understanding of L-Carnitine transport processes in muscle cells as well as in other tissues, and provide pharmacological basis for the treatment of L-Carnitine deficiency.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

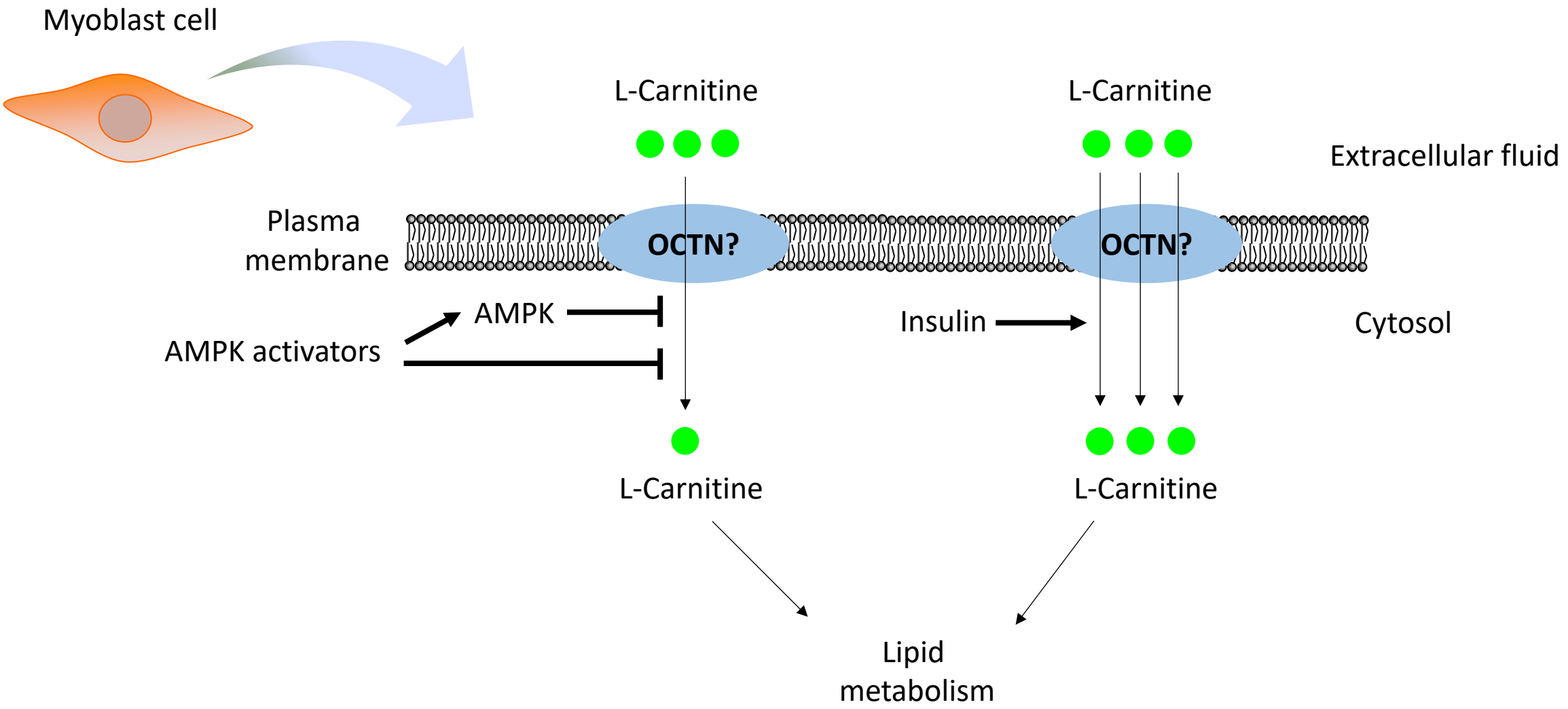
REFERENCES

1. **Banerjee SK, Wang DW, Alzamora R, Huang XN, Pastor-Soler NM, Hallows KR, McGaffin KR, and Ahmad F**. SGLT1, a novel cardiac glucose transporter, mediates increased glucose uptake in PRKAG2 cardiomyopathy. *J Mol Cell Cardiol* 49: 683-692, 2010.
2. **Dzamko NL, and Steinberg GR**. AMPK-dependent hormonal regulation of whole-body energy metabolism. *Acta Physiol (Oxf)* 196: 115-127, 2009.
3. **Flanagan JL, Simmons PA, Vehige J, Willcox MD, and Garrett Q**. Role of carnitine in disease. *Nutr Metab (Lond)* 7: 30, 2010.
4. **Kim M, Shen M, Ngoy S, Karamanlidis G, Liao R, and Tian R**. AMPK isoform expression in the normal and failing hearts. *J Mol Cell Cardiol* 52: 1066-1073, 2012.
5. **Krimi RB, Letteron P, Chedid P, Nazaret C, Ducroc R, and Marie JC**. Resistin-like molecule-beta inhibits SGLT-1 activity and enhances GLUT2-dependent jejunal glucose transport. *Diabetes* 58: 2032-2038, 2009.
6. **Luiken JJ, Koonen DP, Willems J, Zorzano A, Becker C, Fischer Y, Tandon NN, Van Der Vusse GJ, Bonen A, and Glatz JF**. Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. *Diabetes* 51: 3113-3119, 2002.
7. **Pastor-Soler NM, and Hallows KR**. AMP-activated protein kinase regulation of kidney tubular transport. *Curr Opin Nephrol Hypertens* 21: 523-533, 2012.
8. **Shaw A, Jeromson S, Watterson KR, Pediani JD, Gallagher I, Whalley T, Dreczkowski G, Brooks N, Galloway S, and Hamilton DL**. Multiple AMPK activators inhibit L-Carnitine uptake in C2C12 skeletal muscle myotubes. *Am J Physiol Cell Physiol* ajpcell 00026 02016, 2017.
9. **Wilcox G**. Insulin and insulin resistance. *Clin Biochem Rev* 26: 19-39, 2005.
10. **You G, and Morris ME**. *Drug Transporters: Molecular Characterization and Role in Drug Disposition (Second Edition)*. Wiley, 2014.

FIGURE LEGEND

Fig. 1. Schematic Representation of the Roles of AMPK and Insulin in the Transport of L-Carnitine into Muscle Cells. Carnitine is an essential cofactor in the metabolism of lipids and consequently in the production of cellular energy. OCTN: organic cation/carnitine transporter. AMPK: AMP-activated protein kinase.

Fig. 1


Myoblast cell
L-Carnitine
L-Carnitine
Extracellular fluid
Plasma membrane
OCTN?
OCTN?
AMPK
AMPK activators
Insulin
Cytosol
L-Carnitine
L-Carnitine
Lipid metabolism