



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_35088
Title of the Manuscript:	Randle cycle as applied to Diabetes mellitus type 2 'Spruce the basement before dusting the super- structure.'
Type of the Article	Opinion Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	This point of invalidation of Randle hypothesis is difficult for me to appreciate. Look, beta oxidation and glycolysis are catabolic processes but gluconeogenesis is anabolic. Gluconeogenesis does not occur concurrently with glycolysis in the same cell or at least at the same rate so as to prevent the likelihood of a metabolic futile cycle, where no net change in energy is observed due to equal rates of occurrence of the two pathways. Randle hypothesis talks about how the body selectively makes use of fatty acids at the expense of glucose for energy production. What you are claiming to be the basis to invalidate Randle theory is at best a validation of the theory. Indeed, the body will not synthesize energy if it has no need for it. Gluconeogenesis itself requires investment of energy just like other anabolic processes. Hence, if gluconeogenesis is alternating with beta oxidation, it rather confirms the view that energy generation and utilization do not occur at the same time to the same extent. You are comparing an anabolic pathway (gluconeogenesis) with a catabolic one (beta oxidation) here and therefore cannot be said to be invalidating Randle theory/hypothesis. Randle looked at how one substrate is selected at the expense of the other for the same goal of generating energy but by different energy-generation pathways. This underpins the idea of competition for substrates since products of glycolysis and beta oxidation will have to be further oxidized through the citric acid cycle. In your	There are some distortions of what is written in the manuscript and what is commented upon. 1) It was never suggested that the substrate competition is between GN and B-Oxidation. Where as the same is ascribed to the author and went on to say that the author is trying to compare ' GN, an anabolic metabolism with B-Oxidation, a catabolic metabolism. The reviewer goes on to point out the absurdity of competition between them,. Where as no such meaningless suggestion is there in the article. It appears that out of misunderstanding of what is written for which the author is grilled. Let me clarify what is written in the manuscript which is verifiable by any body. A) The substrate competition in mitochondrial matrix is in between the acetyl-CoA produced by glycolysis and the same end product produced by the B-Oxidation. B) The substrate for which competition arises for Oxalo-acetate, which is the first step of the TCA cycle. C) The increased acetylCoA / CoA and NADH/NAD is the result of BOTH glycolysis and B-Oxidation end products ie acetyl- CoA , accumulating in mitochondria. D) The consequence , as per Randle is only PDH inhibition where as, several other enzymes



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	case, gluconeogenesis uses pyruvate as a substrate with beta oxidation requiring fatty acid as the substrate. The two pathways have nothing in common to compete for. To this end, there is no invalidation since the logic differs. Why should pyruvate be re-routed through gluconeogenesis instead of citric acid cycle if there is no inhibition or competition of substrate for the citric acid cycle??? PDH converts pyruvate to acetyl-CoA which would normally enter the TCA. If PDH is inhibited, indeed, pyruvate should accumulate and this condition should favour gluconeogenesis. In this state, per Randles hypothesis, the cell should make use of fatty acid to generate the requisite energy and thus the enhanced beta oxidation. PDH activity is the only way by which glycolytic product enters the TCA cycle via pyruvate, hence, its inhibition is a significant block. How can PDH be inhibited for its substrate to decline in level immediately?? Are you then suggesting the inhibition is due to substrate unavailability? This is problematic especially when you have not presented any novel data to support this opinion. It is interesting to note from your own proposition (table 2) that you agree with Randle regarding the inhibition of PDH by acetyl-CoA and NADH. If PDH is supposed to catalyse the conversion of pyruvate to acetyl-CoA and the enzyme, PDH is inhibited, how can pyruvate level suddenly decline? The immediate decline you are suggesting is logically problematic. Indeed, with time, there is re-routing of the pyruvate into oxaloacetate and thus augmented gluconeogenesis. Under this condition, the initial accumulation of pyruvate would be relieved resulting in its reduced level. Indeed, under the	are also inhibited as illustrated. E) The only effect is inhibition of glycolysis, according to Randle where as the the present hypothesis proves blockage of TCA cycle for both Glycolysis and B-Oxidation end products. The other effects suggested are- low citrate production (isocitrate dehydrogenase inhibition)and citrate liage inhibition resulting in reduced plasma acetyl coA as, for each cittate molecule formed , A mole. Of acty co A is formed , the amount of which determines the stimulation/ or inhibition of ACC / Malonyl CoA 2) It was wrongly inferred and interpreted that the alternation of B-Oxidation and GN as a point of invalidation of Randle hypothesis where as same was foisted by the reviewer on the author and went on to infer, with his own logic that it valdated than invalidated Randle cycle. It is not at all a point of validation or invalidation of Randle cycle by any stretch of imagination. AMPK stimulation starts B-Oxidation where as inhibitin causes GN. Since stimulation/ inhibition of AMPK can not occur simultaneously , it is an obvious fact that B-Oxidation and GN , can only alternate. How can it be ever used by author to invalidate Randle cycle? Yet is ascribed to the author, uncharitably tagged with an acqward comment. Is the proofs abdjuced an invalidation or validation of Randle hypothesis? Yet the teviewer says that what is said only validates Randle hypothesis. What a pity ? Thus the proposed hypothesis is a step forward in offering the reason for preponderance of B- Oxidation over glycolysis and also increased fasting blood
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	condition of reduced pyruvate and CPT1 levels, beta oxidation will be enhanced as you have shown but this does not in any way suggest invalidation of the Randle hypothesis. All that I can understand from your argument is that of reversed conditions necessary for the operation of the Randle cycle/hypothesis. Indeed, Randle never talked about the permanency of the hypothesis he and his colleagues proposed. They stressed the issue of dynamism regarding substrate utilization in energy generation under varied cellular conditions. Randle provided empirical evidence to support the hypothesis. As such, a mere conjecture cannot be used to over-turn such novelty. It is only logical that CPT1 whose increased level inhibits beta oxidation will have the inhibition relieved should the level decline as you have shown. I will strongly suggest that you try and collect data experimentally to prove this idea of yours before presenting it for consideration, please. As things stand now, I have a difficulty in agreeing with you that Randle got it wrong in suggesting that inhibition of PDH leads to accumulation of pyruvate. Indeed, that is what happens but with time the accumulated pyruvate would be re-routed into gluconeogenesis so that the level declines. So the measurement of pyruvate should be properly timed in order not to lead to misleading conclusions. Randle theory/cycle/hypothesis in my view is still valid and in fact validated by your own write-up as what you have depicted is more of what happens during the re-routing of pyruvate.	sugar perpetually, in untreated DM2 . By confusing issues, the reviewer, it is felt, has taken away the credit and handed over discredit. It is regrettable and needs rectification.. 3) The reviewer has maintained that, consequent to PDH inhibition, the pyruvate level will increase which . This is already discussed as case 1 , under the head 'cytoplasmic changes' and also conceded that it is the presently held opinion. The consequences if traced actually stimulated lipogenesis rather than B-Oxidation , against what is contended by Randle. Hence alternative proposal as in case 2 was made . If it is considered that pyruvate level is decreased, the subsequent events lead to what Randle contended ie stimulation of B-oxidation. Hence it was proposed that pyruvate level is decreased consequent to PDH inhibition than increased., a novel idea suggested Besides citing other evidences in favour of low pyruvate , an additional another block was proposed by the present hypothesis at the level of P_k , with good substantiation. Yet the reviewer poses an awkward question as to " how acute decrease in pyruvate occurs consequent to PDH inhibition. Where have the painstaking examples cited gone ? They must have been skipped ! The issue was misinterpreted and questions not relevant are raised.
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<u>Minor</u> REVISION comments	Article requires appropriate editing for improved clarity of grammatical expression. A number of such sections have been highlighted in the main paper for consideration.	
<u>Optional/General</u> comments		