



SDI FINAL EVALUATION FORM 1.1

PART 1:

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 Article:	Opinion Article

PART 2:

FINAL EVALUATOR’S comments on revised paper (if any)	Authors’ response to final evaluator’s comments
Acetyl CoA, the end product of both glycolytic pathway and beta oxidation accumulates in the mitochondrial matrix. When the accumulated acetyl -CoA in the mitochondria exceeds the oxidative capacity of citric acid cycle, feedback inhibition of PDH, through increased ratios of acetyl -CoA/CoA and NADH NAD occurs as contended by Randle. (Is this not proven experimentally? Has it not been established severally that acetyl-CoA inhibits its own further production by inhibiting PDH allosterically??? If this is true, what will happen to the produced pyruvate?? Will it not accumulate?? The accumulated pyruvate can activate the pyruvate carboxylase enzyme to convert it to oxaloacetate. Is this not correct?? But the same increase in these ratios also cause inhibition of AMPK, Citrate synthase, Isocitrate dehydrogenase and citrate lyase and stimulates GN, some of the facts ignored by Randle {How significant are these inhibitions compared to inhibition of PDH by acetyl-CoA and NADH?? That information is pertinent and must be proven experimentally, preferably, under the same experimental conditions of Randle and colleagues before this argument of lack of or absence of substrate competition can be properly accommodated. As already indicated in my comments on the earlier version of this manuscript, Randle cycle hinges mainly on how the cell selects a particular biomolecule for energy generation. Since your contention is that acetyl-CoA from both glycolysis and beta oxidation are blocked from entering the citric acid cycle (CAC), then it presupposes that the CAC itself must grind to a halt due to lack of acetyl-CoA, the substrate required for that important cycle. In effect, energy generation is halted. You need to prove it experimentally. As you are aware, science is evidence-based and not opinion-based without experimental evidence, particularly in this post-genomic era}. The AMPK which is in stimulated in the presence of glucagon excess inactivates ACC by phosphorylation. With inhibition of AMPK now, ACC is dephosphorylated and ACC is active. This increases malonyl -CoA level stimulating lipogenesis, by inhibiting β -oxidation. This is exactly what Randle proposed {No, Randle and co, never made this proposition. This is purely your understanding of their proposition. If they proposed this, why did you make earlier claims that Randle ignored the inhibition of AMPK by the accumulated Acetyl-CoA?? Are you then suggesting here that Randle forecasted correctly and identified all the factors?? In that case, what becomes the basis of your argument??} . The combined effect of inhibition PDH and AMPK is inhibition of glycolysis and β -oxidation, the two major catabolic energy pathways, GN and lipogenesis and the two anabolic path ways are stimulated by AMPK inhibition. {As already indicated, this point is only feasible if both PDH and AMPK are inhibited to the same extent with similar impact on the affected pathways. Only experimental evidence can resolve this, since the extent of inhibition and the exact impact of the inhibition are critical in the acceptance or otherwise of this proposition}. Step 2:- Initiation of GN, lipogenesis and ketogenesis: - The GN and ketogenesis sustain the energy supply to vital organs like brain when	



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both the major energy producing pathways are inhibited temporarily. – {Remember GN and ketogenesis themselves only provide the substrates for energy generation. As such, their products must be oxidized through pathways that generate energy. In fact, both anabolic processes rather consume energy without producing net energy.}

Ketogenesis:- Since the accumulated acetyl -CoA cannot enter the citric acid cycle, the flux of β –oxidation which is already in mitochondrial matrix, is re -routed through ketogenic pathway. This produces acetone (and other ketone bodies) ultimately, capable of crossing blood brain barrier to reach the brain, to sustain its activity. They also serve as metabolic fuel for other tissues which can utilize them as source of energy. This explains the facts that ketogenesis accompanies sometimes GN. They are also responsible for glucose sparing effect when glucose is in short supply. {So under this condition of glucose sparing, how does the cell generate energy to sustain its activities?? What do you even mean by glucose sparing?? If glucose is to be spared, what biomolecule will be the reliable source of energy??? Is it not fatty acids from fat breakdown?? Now, if fat is to be oxidized is it not done mainly through beta oxidation?? What is the source of the acetyl-CoA used in ketogenesis?? Can it be from beta oxidation?? If beta oxidation is shut as you have suggested, how will acetyl-CoA be obtained to produce the ketone bodies???

Acetate appears to be the substrate of choice in starvation for synthesis of FA (Under starvation, do you not think that the cell will rather find a means of generating energy via lipolysis to give the FA for oxidation?? If FA must be synthesized from acetate, it must occur under fed state where the cell has generated more than enough energy for the excess to be channeled into anabolic activities like FA synthesis from acetate. Do you not think that under starvation, acetate should rather be converted to acetyl-CoA for further oxidation through the citric acid cycle instead of being used for FA synthesis??

The increased acetyl-CoA levels allosterically inhibits citrate lyase enzyme and prevent the entry of acetyl -CoA into the cytoplasm. It is the cytoplasm where FA synthesis takes place.{Look, what you are portraying here rather should occurs under fed state NOT starvation. Remember, you just said FA is to be synthesized from acetate, so where does your acetyl-CoA come from to induce this allosteric inhibition of citrate lyase?? So, you have acetate and acetyl-CoA accumulating under fasting state to be used to synthesize FA in the cytoplasm???? Where is your evidence???? As indicated already, this is really difficult to accept. You need to provide experimental proof as the state of the art requires that acetate, should rather be activated to acetyl-CoA to allow for entry into the citric acid cycle for generation of the required energy to meet cellular demand under starvation.}

Re -activation of β -oxidation and its perpetuation:-

The cytoplasmic acetyl CoA and the NADPH, both being substrates for ACC in Conversion of pyruvate to malonyl –CoA by ACC, their reduced levels inhibit ACC. Malonyl-CoA is consequently reduced which leads to stimulation of β -oxidation. It may be noted that this stimulation of ACC is brought about independent of AMPK (by low acetyl CoA level inhibiting ACC) which continue to inhibit PDH. Thus while β -oxidation is restored, glycolysis is still under inhibition, initially by PDH and perpetuated later by PDK 4. Also the citrate produced thus partly removes the inhibition of ACC by AMPK, allosterically, through forward loop stimulation, which further augments the passage of β -oxidation products through the citric acid cycle.[10] On the other hand, the LCFA inhibit ACC through negative feedback regulation, through phosphodiesterasae induced inhibition of c AMP and PK, favoring β –oxidation.[11] At transcription level, ACC is regulated by SREBP I C through insulin and by ChREBP by high carbohydrate diet. Once the β - oxidation



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cycle starts, the GN stops. The GN pathway remains shut off until the end of β - oxidation cycle and cellular energy level indicators are high. When the cellular energy levels are high, β -oxidation is shut off by the inhibited AMPK, and simultaneously the GN begins. Thus the β - oxidation cycle and gluconeogenesis cycles alternate while glycolysis is inhibited {What really stops GN when beta oxidation starts?? Where is the experimental evidence??? What inhibits the glycolytic process? Why is beta oxidation perpetuating with inhibition of glycolysis if there is no competition for entry into the citric acid cycle as suggested by Randle?? What really prevents glycolysis from also taking place??? Is it not due to the glucose sparing for use by cells that cannot rely on beta oxidation for energy generation?? Is this not the crux of Randle's hypothesis???? What is new if anabolic pathway like GN alternates with a catabolic type like beta oxidation??? Are you suggesting that both GN and glycolysis remain shut until beta oxidation alone takes place till the cell is satisfied??? How is that possible?? Do you not think that what Randle talks about competition between acetyl-CoA from beta oxidation and the one from glycolysis is what you are misinterpreting it to mean alternation of beta oxidation with GN?? Look, Randle and co emphasized that the selective acceptance of acetyl-CoA from beta oxidation into the citric acid cycle by the cell causes accumulation of acetyl-CoA from PDH which in turn inhibits the PDH allosterically, so that pyruvate, the product of glycolysis is also accumulated. The accumulation of pyruvate also inhibits key glycolytic enzymes while favouring gluconeogenic enzymes for gluconeogenesis to occur. As such, glucose is generated to be used by cells that rely solely on glucose for energy. How can cells that cannot obtain energy by beta oxidation get their energy if both glycolysis and gluconeogenesis are shut??? This does NOT occur. Even in senescence, there are still some metabolic activity}.

This explains the continuation and perpetuation of β -oxidation suppressing the glycolysis without there being any substrate competition, thus invalidating the substrate competition theory of Randle. {Your argument about alternation of beta oxidation with GN is interesting. What is common between the two pathways such that one must necessarily wait for the other to complete??? Any common enzymes, substrates or intermediates??? What really holds one in check for the other to complete??? Where is the evidence to this assertion??? Why not GN and glycolysis which have several things in common??? Are you by any stretch of imagination implying that when fatty acid is broken down to generate energy, GN and glycolysis are halted till complete cessation of beta oxidation??? In that case, both GN and glycolysis must begin as soon as beta oxidation is shut off!! But that is not the case since the likelihood of futile cycle is very high. Provide experimental proof of this proposed shutting off of GN and glycolysis during beta oxidation!!! As far as I am concerned, that is unlikely. Perpetuation of beta oxidation only implies the need for more energy. As such, both glycolysis and beta oxidation ought to be activated based on specific cellular needs. Some cells can depend on beta oxidation whereas others rely on glycolysis for their energy. Cells that cannot make use of product of FA breakdown for energy generation must meet their energy requirements mainly via



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glycolysis. Remember, carbohydrate storage is not as high as lipids. This is where further oxidation of pyruvate to acetyl-CoA is inhibited to spare the glucose for cells that solely rely on it for energy generation. And this is where the issue of competition comes in, otherwise, there will be nothing called sparing of glucose. As such, glucose synthesis is rather encouraged as proposed by Randle. Remember, Randle and colleagues did not just speculate, rather, they proved their concept with experimental data. All that you are proposing is not backed by any data, yet you claim to be invalidating an evidence-based theory. How is that possible?? Once again, you have not and are not invalidating anything. What you are proposing here is not supported by any verifiable experimental data. Science again is not based on speculations and conjectures. You must prove your proposal with experimental data first before attempting to attack any evidence-based theory. For more information on the Randle issue and how it operates under varied conditions, let me please, refer you to this review paper

[Louis Hue](#) and [Heinrich Taegtmeyer](#). The Randle cycle revisited: a new head for an old hat. [Am J Physiol Endocrinol Metab](#). 2009 Sep; 297(3): E578–E591.

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