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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	Comment-1: - 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?? Reply:- What is written is just a restatement but not repudiation of what Randle stated about PDH. The remarks appear to be out of context as nothing written there in was disagreed including the increased ratios pointed out or stimulation of enzyme PC., here or any where in the article. Even the matter is further clarified under sub heading " The Gn". Even your question on pyruvate has been extensively dealt with as to whether it increases (present view)or decreases(proposed hypothesis) in cytoplasm backed by good substantiation.. The remarks appear to have been made not after going through the article fully but after peicemeal reading, as there is no scope for such valley of questions other wise . What follows below your remarks is, but an extension of Randle cycle, by way of facts - not taken into consideration by original hypothesis of Randle or even by his subsequent reviewers. This is one major contribution of this hypothesis in reinterpreting Randle cycle. Hence the comments are requested to be withdrawn.. Comment-2: - 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, Reply: - The inhibition of these enzymes is very important because they block



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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 phosphodiesterase 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

TCA cycle as already pointed out. This makes neither the acetyl coA from glycolysis nor B- Oxidation to enter the TCA cycle. This initial locking of TCA Cycle is momentary as it is immediately unlocked by events that restart and perpetuate B oxidation , with glycolysis inhibited at the same time. In this hour of energy crises metabolism of vital organs is sustained by the ketone bodies that are in mitochondria but denied entry into TCA. Subsequently the energy metabolism is solely managed by B oxidation- this being what is meant by perpetuation of B oxidation.

Comment-3: -

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. 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.

Reply -.

Out of many propositions by Randle, what you call “Randle cycle hinges” is but one aspect the selection and adaptation to metabolic demand is of available substrate is but one aspect. For example a contracting muscle using FA as fuel after glucose is exhausted. This aspect is not the bone of contention but substrate competition in MM. The enzymes enlisted are being additionally inhibited by the same increase in ratios as Randle considered in case of PDH. A little verification, is sure to clarify this misunderstanding. Because The references was made only after thoroughly verifying the involvement of each enzyme, but not out of fanciful imagination. Then you would agree perhaps that what is written is 'evidence based' and 'facts accepted in literature need not be again verified experimentally'. The additional information focused in this article might have come to light subsequent to the original publication in 1963 of Randle cycle , hence possibly not reflected in his original publication. . But there is no point in missing them by the subsequent as well as the latest reviewers of Randle cycle- spanned upto 2016.The present work fills that void.

Comment-4: -

No, Randle and co, never made this proposition. This is purely your understanding of their proposition.

Reply-

There is a typing omission in the statement b, that could have given erroneous impression. Corrected version reads thus as " This exactly is the "opposite "of what "subsequent reviewers of Randles cycle "tried to explain.



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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 glyconeogenesis 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

Comment-5: -

If they proposed this, why did you make earlier claims that Randle ignored the inhibition of AMPK by the accumulated Acetyl-CoA??

Reply-

What is said is true. Neither Randle nor subsequent work suggested AMPK inhibition besides PDH inhibition. It is the original contribution of this present work .

Comment- " R u suggesting that... "

Comment-6: -

Are you then suggesting here that Randle forecasted correctly and identified all the factor?? In that case, what becomes the basis of your argument??

I vehemently deny making ay such suggestion. It is your are interpolation. and wrong inference of what is not said. How can u expect that I would say something that goes against my hypothesis? The question is mischievous!

Comment-7: -

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.

Reply- This seems to be only your assumption, not backed by any reference or experimental proof. Hence, my inability to accept or answer to your above remark.

Comment-8: -

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.

Reply-

This has been aptly and amply replied in subsequent answers...

Comment-9: -

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.



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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.

Reply-

Liver, muscle, heart, brain and kidney have glycolytic enzymes to produce glycolysis . Liver is the source of whole body energy metabolism where as the product of glycolysis muscle,, brain and heart the energy is used locally. Brain and heart having GLUT 1 transporters can extract plasma glucose even at low levels. But Liver and muscle need higher plasma concentration to keep going their glycolysis. So at low glucose levels noth liver glycolysis, and muscle glycolysis stops, but that of est of tissues depend on pproduced by glycolysiscor B oxidation.ATP vital organs like brain and heart, continue. If still glucose level is low or metabolic demand high, they can utilize ketone bodies as energy sources. How the do it using these fuels is beyond the scope of this article. G N and ketogenesis being anabolic use energy and there is no doubt about it. The energy levels built up by glycolysis (in fed state) and B oxidation (in fasting) is used for these energy requirements. The block of both metabolisms conceived is just temporary until perpetuation of B oxidation occur to meet the energy demands of these two energy consuming processes. During the said transient block vital organs are fed on ketone bodies at the expense of other tissues.

Comment-10: -

So under this condition of glucose sparing, how does the cell generate energy to sustain its activities??

Reply:-

This is already answered above.

Comment-11: -

What do you even mean by glucose sparing??

Reply-

"glucose sparing is a physiological response to save the functioning of vital organs like brain and heart When glucose supply is less or metabolic demand is high (as in ac M.I) by substituting energy requirements through ketone bodies as fuel instead of low glucose- this is called glucose sparing action of fats. It operates only in energy crisis only and is only a temporary adjustment during reorganization of energy metabolism.

Comment-12: -

If glucose is to be spared, what biomolecule will be the reliable source of energy???

Reply-

As already seen above , the biomolecule are ketone bodies.



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	Comment-13: - Is it not fatty acids from fat breakdown?? Now, if fat is to be oxidized is it not done mainly through beta oxidation?? Reply- it is from acetyl co a , accumulated in MM and is initially barred from entering TCA cycle as seen above. It is only temporary till energy crisis is tide over by subsequent induction of B oxidation alone when its acetyl coA can negotiate TCA as initial conditions of increased acetyl coA/ coa don to exist .so there is no need to depend on B oxidation of fats to produce acetyl coa. Comment-14: - What is the source of the acetyl-CoA used in ketogenesis?? Can it be from beta oxidation?? If beta oxidation is shut as you suggested, how will acetyl-CoA be obtained to produce ketone bodies??? Reply- already hiven above .it is the trapped acetyl co A in MM consequent to transient shut down of TCA cycle initially.. Comment-15: - Under starvation, do you not think that the cell will rather find a means of generating energy via lypolysis 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?? Reply- Acetate as source of acetyl coA in starvation has umpteen number of references in literature. This is because the best source- the fats are scarce as lipogenesis is defective in fasting(pl see references. In manuscript). This acetate is used to produce cytoplasmic acetyl CoA which goes into lipogenetic pool through ACC- melonyl coA - CPT 1 axis . It does not enter mitochondria as to go into B oxidation as questioned by you. On the other hand the acetyl coA that is produced in mitochondria remains in mitochondria to go into B oxidation. Confusion seems to be in recognizing the respective roles of cytoplasmic and mitochondrial variants of acetyl co A and their respective distained courses. Just to clear any further doubt how
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	these two acetyl coAs remain at designated places is clarified that acetyl coa of B oxidation is produced in mitochondria itself and that produced from glycolysis is also produced in mitochondria because PDH , the producer is also in the mitochondria !. The cytoplasmic acetyl coa is produced as a result of citrate shuttle from mitochondria to cytoplasm the amount of citrate that shuttles determines the amount of cytoplasmic acetyl co A. All this has been clearly discussed in the article but appars to have not followed closely , as inferred by the nature of questions shot. Comment-16: - Look, what you are portraying here rather should occur 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 by starvation. Reply- The answer is very much there in the manuscript itself. The acetyl z CoA which causes increased acetyl-coA/ coa ratios that inhibits PDH is also responsible for the inhibition of citrate lyase and all other extra enzymes pointed out by the author in addition to PDH pointed out by Randle and co. The change in explanation to the mechanism under consideration because the new facts can not be ignored. Randle himself can not be held responsible if his concepts need to be modified in view of subsequent developments. A number of original contentions of Randle have been disproved the brief reference is given in the introduction . It is not first time his contentions have been differed (as shown in introduction) but thus ' substrate competition ' mechanism has for the first time here and now, after nearly half a century after it is proposed. So this sensitive issue needs to be compassionately examined as to its veradity than eighth contempt. None is exempt from correction and modification- be it is Dalton (and his atomic theory) or Randle (his substrate competition theory. Here the personalities are not as important as facts. Your belief that all contentions of Randle are experimentally proved some of the enlisted rejected contentions as enlisted in introduction are mere postulates that subsequent experiments by others, not Randle, have invalidated them. Now any hypothesis is only proposed and from all sides it is verified if there is merit, but not by the proposer himself. Take the example of Dalton or Socrates- they only hypothesized and later verified by others.
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	Then why do you look at my hypothesis differently? And insist that I should not talk without experimental verification? PI note that it is not an ' opinion on Randle cycle' but had to stoop to get published as ' opinion type article, as there is no scope for ' bypoghesis type in almost all journals. This precludes new and novel ideas from seeing the light as such articles fon ot fit into any other format and hence subject to rejection. Comment-17: - What really stops GN when beta oxidation starts?? Where is the experimental evidence??? Reply- The anabolic , GN, and Catabolic . B oxidation can't go simultaneously. The master metabolic switch, the AMPK.when switched on, stimulates B oxidation and when switched off , stimulates GN .obviously , the swith can be on and off at the same time. Comment-18: - What inhibits the glycolytic process? Reply- This has been answered in the manuscript clearly. First it is PDH and later PKM2 which is active under conditions of starvation etc. Comment-19: - Why is beta oxidation perpetuatingwith inhibitionof glycolysis if there is no competition for entry into the citric acid cycle as suggested by Randle?? Reply-. B oxidation perpituates in starvation under the inflence of glucogon. Compitition or no compition from glycolysis - untill it is trminated by insulin if reffeding starts, in starvation , In DM2 when controlled with insulin controlled or interdigestive phase, when it is terminated by food intake again. Comment-20: -
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	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???? Reply- THE vital organs like BRAIN AND HEART ,since they can extract glucose even in low levels sustain their metabolism even under low BS levels .But when metaboli demand is more , they use ketones as fuel and this effect is called the glucose sparing effect of fats. Jin the initial stages when both energy metabolisms come to stand still briefly the ketones amd GN sustains the vital organ .Soon ♦♦ B oxidation starts by mechanism other than stimulation of AMPK as hypothesised. If AMPK IS INVOKED IT WOULD STIMULATE GLYCOLYSIS AND THE ORIGINAL DRAMA INC IS REPEATED. .TO UR CONCEPT OF GLUCOSE SPARING IS ALSO NOT CORTRCT .WHEN GLUCOSE SUPPLY IS LESS AND METABOLIC DEMAND IS MORE THE ADDITIONAL ENERGY IS MET BY KETONE BODIES.THIS IS GLUOSE SPARING . GLCOSE SPARING IS AN EMERGENCY MEASURE OCCURING IN PATHOLOGICAL BACK GROUND WHERE AS RANDLE CYCLE TALKS OF NUTRIENT AVAILSABUILITY AND METABOLIC ADAPTATION. RESTING MUSCLE USES GLUCOSE FORENERGY AND UNDER EXCERCISE IT SEITCHES ON TO B OXIDATION FOR ENERGY AS THE SUPPLY FROM FORMER ARE LIMITED. Comment-21: - What is new if anabolic pathway like GN alternates with a catabolic type like beta oxidation??? Reply. It is not the question of being mew, the mechanism which keeps the alternating wthout involving simultaneously glycolysis and B oxidation(which would cause braek down of TCA cycle). is the crux of his hypothesis Comment-22: - 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?? Reply- Untill fasting is terminated The B oxidation is the only enegy supplier and glcolsis has got to just stop (imagine how glycolysis can function
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	when hlucogon/ insulin ratio is high in starvation?) Comment-23: - Your argumentof 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??? Reply- it is already explained that B O and GN have to alternate only because they are controlled by the master switch AMPK, WHICH IN ON POSTION STIMULATES B O AND OFF POSITON STIMULATES GN, AS SUCH WHOE THE SWITCH CAN BE BOTH OF AND ON AT THE SAME TIME ? THE ALTERNATION IS JUST BECAUSE OF THIS AND NOT BECAUSE OF ANY COMMON ENZYME/ SUBSTRATE U R TALKING ABOUT. The evidence u demand is in all text books of biochemistry and in all articles dealing with AMPK. IT IS SURPRISING HOW IT HAS ESCAPED UR ATTENTION. Comment-23: - 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??? Reply- This is just fundamental.glycolysis whose enzymes depend on insulin can not operate under B oxidation controlled by glucogon, both hormones being opposite in action. So glcolysis should stop when B oxidation is going on. It doesnot need experimental proof. Just referal to the relevant subject is enough. Comment-24: - !! But that is not the case since the likelihood of futile cycle is very high. Provide experimentalproof of this proposed shutting off of GN and glycolysis during beta oxidation!!! As far as I am concerned, that is unlikely. Reply- It is stressed that what is said by me is "while glcolysis is ihibited, B-
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	Oxidation and G N alternate (how they alternate under influence of AMPK is already explained).But u say that glycolysis and GN if started immediately , the chances of utile cycle are high. It is ur interpolation. I once again re-assert that under stipulations of my proposed hypothesis, no futile cycle occurs as both GN and glycolysis do not occur similtaneous. All this is a FUTILE EXERCISE" -Your contension is wrong because the perpetuation of BO in starvation is not because of increased energy need but because the dominance of glucogon is unchallenged by insulin. Re -feeding in starvation makes insulin dominant over glucogon and consequently, glucolysis predominates over B O. Likewise B- oxidation prevails in interpranidal state under influence of glucogon which is reversed to glycolysis when inslin secretion becomes plenty in pranidal state. Normally under physiological conditions, perpituation of B oxidation does not occur because of interweaning fed state but in starvation since there is no interweaning fed state, the B O , Continues uninterrupted . Similar is the case with un -treated DM2, till treated and euglycemic state is achieved. In this context only what is ssid of , Comment-25: - 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. Reply- The perpetuation of B oxidation is becaue under condions where it is cited to occur, ie in starvation and uncontrolled DzM, it is because glucogon dominates as energy producing mechanism without any break for insulin to start glycolysis- unlike in inter digestive period. Though,B oxidation prevails when once feeding starts, glycolysis starts under infl uence of insulin. Thus only under nomal physiological state cells get energy foom both metabolisms that too in alternation only. So your concept of perpetuation of B ocidation is not correct. THERE IS NO SCOPE FOR CELLS FOR RECEIVING ADDITIONAL DOSE OF ENERGY FROM GLYCOLYSIS AS suggested by you BECSUSE GLYCOLYSIS CAN NEVER OCCUR AS LONG AS GLUCOGON DOMINATED FASTING IS CONTINUED. Comment-26: - . Cells that cannot make use of product of FA breakdown for energy generation must meet their energy requirements mainly via 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 competitioncomes in,
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otherwise, there will be nothing called sparing of glucose. As such, glucose synthesis is rather encouraged as proposed by Randle.

Reply-

All cells (except those like brain etc) can't use even if glucose is supplied as contended by you, because of lack of glycolytic machinery. So, they use ATP
Irrespective, whether from glycolysis or B- oxidation.

Comment-27: -

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 impossible, at least, until you support it with verifiable experimental data.

Reply-

I don't agree on this point. Randle cycle originally, is a mixture of experimentally verified truths and some speculations. There is no denial of this point, as the contentions disproved subsequently (as enlisted in the introduction) are all speculations, which on subsequent experimental verification are disproved. Just to cite from the list of rejected speculations of Randle are "inhibition of hexokinase and consequent Failure of glucose to enter into glycolytic cycle" ..under conditions contemplated by Randle. The hexokinase inhibition was rejected, as the same did not occur under conditions of experimental verification. Likewise his speculation of inhibited hexokinase as the cause of failure of glucose to enter glycolytic pathway is rejected in favour of the fact that "failure of translocation of GLUT -4 is the cause of non-entry of glucose"
As Randle cycle is looked upon as being 'above board' and any attempt to comment on it is 'scoffed at', the following inferences are deemed to be necessary to "put the matter in proper perspective".
1) That, even Randle hypothesis has some speculations.
2) INFACT they were experimentally verified subsequently. some of Randle's contentions were accepted, some rejected.
3) His hypothesis was not rejected, as no experimental proof was provided. This in fact is the rule, than exception, when any new hypothesis is made, be it by Randle or Dr Prasad! Any hypothesis is but a 'bundle of facts' to be confirmed later"



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	Comment-28: - If you agree to the inhibition of PDH, why are contesting the accumulation of pyruvate as a result of the PDH inhibition? Reply- I accept PDH inhibition as it is universally accepted fact. Why I contest increased pyruvate in plasma following PDH inhibition is because it does not lead to preferentially establishing B O as proposed by Randle but the opposite ie lipogenesis occurs as depicted in the illustration and text in the manuscript. on the other hand it is also shown that if low level of pyruvate is considered , the intended result of Randle ie B- Oxidation occurs. So unbiasedly both cases (1 & 2) in the manuscript are considered and also by the extensive evidence in favour of low pyruvate is also adduced. Ofcourse it is daring to go against the prevailing opinion, but not after establishing that the present view doesn't deliver the goods and evidence exists against it. Comment-29: - Are you by any means suggesting that glycolysis stops abruptly just because beta oxidation has started??? Reply- The conditions that favour B oxidation (glucagon) when gain upper hand over conditions that don't favour (insulin) at that moment the B -Oxidation starts. No where it is mentioned by me that it suddenly changes or that any perticular effect is ascribed to it. It is just ur interpolation to defeat the purpose of this heypothesis. Comment-30: - You are assuming erroneously that any increase in acetyl-CoA level should necessarily activate ACC. Why that assumption?? Have you consideredthat different enzymes can respond differently to a change in concentration of a given substrate due to varied affinities for the substrate as described by K_m values? Above all, cellular conditions are not static but very dynamic and any attempt to assume permanency of conditions could lead to wrongfulinterpretation. Reply- My view on the effect of concentration of the substrate acetyl coA , on enzyme ACC , is not erronious. as u call it, If enzyme chemistry is reviewed, the effect of increasing substrate on the concentration of the product is grapbically stated. In fact the superiority of the effect of substrate on the enzyme over other inflences like allosteric stimulation/ inhibition is also clearly stated. It is true that when there
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	are two reactants of which concentration of one atleast is increased, the system balances by increasing the quantum of product produced as per kinetic studies of enzymes.. So the substrate vs enzyme cocept is invoked after exhaustive examination of enzyme kinetics but not as a fanciful idea, as might have been thought.. Your contensions that different enzymez have different affinities, Kms ETC is not relevant here because we are dealing with one enzyme only and not comparing with any other enzymes . So, matters raised in this regard are irrelevant to the context . Comment-31: - <u>Need for experimental proof of the hypothesis as again and again commented by reviewer.</u> <u>Reply:-</u> If a hypothesis Is sound, it is indipendently investigated and if necessary verified by experiments and after that only the hypothesis is accepted or rejected. But why in this case experimental proof is sought again and again even before the hypothesis is accepted for publication ? If any postulates are not at all accepted without experimental proof,,half of the Randle hypotheses are based on speculations (the proof is that some of them are rejected after subsequent experimental verification, as enlisted in introduction) - It would not have been possible for the Randle Cycle to see the light. Why the bias here ? 4) The points that could be varified experimentally to validate or invalidate this hypothesis are given at the end of the paper. To be able to distunguish between what is a scietifucally accepted fact and what is the newly proposed idea, is very important- both do not need experimental proof, it may be noted. when some of Randles contensions could be disproved subsequently ,(as deslt with under introduction part) why it is not possible, that more could follow the suit ?... " Seek and Ye shll find"- is the guiding principle/motivation for the present work. The present hypothesis added a new dimentions to Randle hypothesis of substrate compitituton and a new mechanism for preponderance of beta oxidation over glycolysis put in view of further adduced evidence .put Is it undesirable? why look at Randle cycle as Bible inspite of already exposed holes in it and why scorn at another hypothesis that tries to 'comment on another possible hole ' which went unchallenged for nearly 5 decades ?. " Who said what, - is not important , what is said by any".. is important, provided there is sufficient reason to believe."
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