Cancer cachexia has many symptoms but only one cause: anoxia [version 1; peer review: awaiting peer review]

A hypothesis

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Abstract
During nearly 100 years of research on cancer cachexia (CC), science has been reciting the same mantra: it is a multifactorial syndrome. The aim of this paper is to show that the symptoms are many, but they have a single cause: anoxia.

CC is a complex and devastating condition that affects a high proportion of advanced cancer patients. Unfortunately, it cannot be reversed by traditional nutritional support and it generally reduces survival time. It is characterized by significant weight loss, mainly from fat deposits and skeletal muscles. The occurrence of cachexia in cancer patients is usually a late phenomenon. The conundrum is why do similar patients with similar tumors, develop cachexia and others do not? Even if cachexia is mainly a metabolic dysfunction, there are other issues involved such as the activation of inflammatory responses and crosstalk between different cell types. The exact mechanism leading to a wasting syndrome is not known, however there are some factors that are surely involved, such as anorexia with lower calorie intake, increased glycolytic flux, gluconeogenesis, increased lipolysis and severe tumor hypoxia. Based on this incomplete knowledge we put together a scheme explaining the molecular mechanisms behind cancer cachexia, and surprisingly, there is one cause that explains all of its characteristics: anoxia. With this different view of CC we propose a treatment based on the physiopathology that leads from anoxia to the symptoms of CC. The fundamentals of this hypothesis are based on the idea that CC is the result of anoxia causing intracellular lactic acidosis. This is a dangerous situation for cell survival which can be solved by activating energy consuming gluconeogenesis. The process is conducted by the hypoxia inducible factor-1α. This hypothesis was built by putting together pieces of evidence produced by authors working on related topics.

Keywords
Cancer cachexia, anoxia, intracellular lactic acidosis, weight loss, nutritional supplements, quality of life, Cori cycle, dichoroacetate, emodin, pentoxifylline
Introduction
Cancer cachexia (CC), also known as cachexia-anorexia syndrome\(^1\), is a consequence of cancer in which patients lose weight with an overall decline in health\(^2\). It is a combination of starvation and metabolic disturbances\(^3\) that greatly affects quality of life disrupting regular daily activities. In the more advanced states, cancer anorexia-cachexia cannot be modified or improved by increased feeding, stimulating appetite, or nutritional supplements\(^4\).

The cardinal clinical points of this syndrome are\(^5\):
- Progressive and relentless weight loss (more than 5% of loss compared with normal weight).
- Loss of muscle mass.
- Loss of fat tissue.
- Minimal or no response at all to usual therapies such as nutritional supplementation.
- Difficulties in routine daily activities.
- Marked fatigue or asthenia.
- Loss of appetite.
- Progressive deterioration.
- Reduced cancer treatment tolerance.
- Reduced length of life. (There is a correlation between weight loss, rate of weight loss and survival.)
- Anemia.
- The reduction in food intake alone does not explain the metabolic changes found.
- Resting energy expenditure (basal metabolic rate) is increased in some cancers but not in others that also produce cachexia.
- Associated with insulin resistance.
- Associated with acute-phase inflammatory reactants.

In advanced phases of cancer up to 80% of patients exhibit CC\(^6\). Complex associations between cancer, host, nutrition, psychological, systemic and environmental factors were thoroughly studied as part of the problem. However, a unified and physiopathological explanation is lacking. This led to consideration of CC as a multifactorial consequence of cancer. Based on the thousands of publications and findings on CC, it is the objective of this work to arrange the multiple pieces of evidence published in the world medical literature and to build a comprehensive and unified picture of the syndrome. Understanding how anoxia, rather than hypoxia, is the main culprit of CC will allow for a different approach to treatment on a rational basis. A certain amount of speculation is involved in the proposed solution of the puzzle; however, this speculation has been kept to a minimum. Only laboratory work and clinical trials can ultimately confirm the validity of the hypothesis developed here.

Known mechanisms of cancer cachexia
As already mentioned, CC is described as the consequence of cancer-induced loss of appetite (reduced food intake-starvation) and metabolic alterations. It is found frequently in advanced tumors and many authors consider it a paraneoplastic syndrome\(^6\). Many mechanisms contribute to unleashing cancer cachexia. Some are known, others are not. However, a superficial look may be misleading because the problem is far more complex than it may seem. There are well known factors that play a role, which are summarized below.

Lack of appetite: reduced food intake
Although this may seem to be the main cause, it is not. Pharmacologically improving the appetite or increasing food intake does not solve the problem. Usually, it does not stop progressive weight loss. Anorexia is frequent, but there are many patients who lose weight without a manifest loss of appetite. Important contributory factors to anorexia are depression\(^8\), and cancer treatments themselves, such as chemotherapy and radiotherapy\(^9\). However, this treatment-related weight loss does not seem directly associated with cancer cachexia.

Inflammatory and catabolic mediators
These mediators were found increased in cachexia, such as tumor necrosis factor alpha (TNF\(\alpha\))\(^10\–\)\(^14\), ZAG (Zinc-\(\alpha\)-2-glycoprotein also known as lipid mobilizing factor)\(^15\),\(^16\), inter-leukin (IL)\(-\)1\(^\alpha\), IL-6\(^\alpha\), IL-15\(^\alpha\), proteolysis inducing factor (PIF)\(^17\), myostatin\(^18\), and transforming growth factor-\(\beta\) (TGF\(-\beta\))\(^19\), among others. Levels of glucocorticoids are also increased. These mediators seem to be part of the problem but not the cause. For example, TNF\(\alpha\) and IL-6 produce loss of appetite by interacting with hypothalamic receptors that regulate food intake\(^20\). However, steep elevation of IL-6 is found mainly in very advanced stages of CC\(^21\). IL-6 is also involved in an autophagy inducing activity found in serum of patients with CC. Blocking IL-6, this activity disappears\(^22\).

Increased energy expenditure (higher basal metabolic rate)
A previous study showed that 143 out of 297 (48%) unselected cancer patients exhibited increased resting energy expenditure\(^23\). Several authors have confirmed increased energy expenditure as a cause of weight loss in cancer patients\(^24\–\)\(^29\). This probably is the consequence of increased uncoupling at the electron transport chain and increased Cori cycle activity. In the liver, the excess lactic acid produced by the tumor can be converted into glucose (Cori cycle) consuming ATP. The Cori cycle is considered an important culprit in cachexia. The Cori cycle (also known as the lactic acid cycle), converts lactate produced by anaerobic glycolysis in muscles to glucose in the liver. In cancer, instead of muscle, the tumor is the provider of lactic acid. While glycolysis produces a positive balance of two molecules of ATPs, the Cori cycle uses up six molecules of ATPs. Each turn of the cycle represents a net loss of four ATP molecules\(^30\). The Cori cycle has been held responsible for energy loss in cancer by many authors\(^30\–\)\(^33\). An intracellular Cori cycle cannot be ruled out as the main cause of energy loss (Figure 1).
Figure 1. The Cori cycle and glycolysis. The figure shows that six ATP molecules are necessary to convert two molecules of lactate into glucose (left panel). On the other hand, the glycolytic pathway (right panel) produces two ATP molecules by degrading glucose to lactate. If two molecules of lactate produced through glycolysis are reconverted into glucose, there is a net loss of four ATP molecules. If this is established as a permanent circuit glucose-lactate-glucose circuit, each turn in the circuit loses four ATP molecules. Interestingly, the Cori cycle is activated during fasting where it contributes to generating glucose. Patients with advanced metastatic cancer show an increased Cori cycle, particularly those patients with high glycolytic flux.

According to our criteria, the Cori cycle by itself, is the main responsible actor in CC, but not the originating cause. Figure 2 and Figure 3 show a proposed mechanism of how the Cori cycle develops in cancer and the energy imbalance it drives. For each molecule of glucose produced through the Cori cycle, six molecules of ATP are used. For each molecule of glucose degraded to lactic acid only two molecules of ATP are produced. Therefore, if a cycle is established in which one molecule of glucose produces two ATP molecules, and the lactic acid thus formed is used to regenerate glucose, four molecules of ATP are lost in each complete cycle.

Loss of adipose tissue
Loss of adipose tissue due to increased lipolysis seems to be activated by protein kinase A. Hepatic nuclear factor-4 (HNF4) mRNA was downregulated in adipose tissue of patients with CC. Degradation of triglycerides also has a role in the loss of adipose tissue with the intervention of adipose triglyceride lipase. ZAG (lipid mobilizing factor), which decreases lipids from adipocytes, also increases the expression of uncoupling proteins in adipose tissue and skeletal muscle and therefore produce potential energy loss. Interestingly, the expression of ZAG is increased with hypoxia and induces insulin resistance.

Loss of skeletal muscle
Loss of skeletal muscle occurs due to increased proteolysis and decreased protein synthesis.

Tumor stage
Tumor stage seems to be a predictive factor of cancer cachexia and is probably related to tumor mass.

Insulin resistance
Asp et al. found that CC bearing mice had a significantly decreased glucose response to insulin. Rosiglitazone improved insulin sensitivity. Muscle wasting seems to be also related to insulin resistance. HIF-1α can induce insulin resistance. Intermittent hypoxia has the same effect.

Tumor bioenergetics
If we look at tumor bioenergetics as a highly dynamic process that constantly adapts metabolism to oxygen and nutrients availability, we understand that most cancers have three types of cells according to their glucose metabolic behavior:

a) Oxidative when oxygen availability is high (normoxic behavior).

b) A variable mix of glycolytic and oxidative with or without hypoxia. The Warburg effect in this case is the preference for glycolytic rather than oxidative pathway.

c) Fully glycolytic when oxygen is absent (anoxic behavior).

These three types of metabolic behavior may be present in the same tumor and vary in proportion as the tumor progresses. In very advanced tumors or very bulky ones, anoxic behavior predominates and causes CC.

Figure 3 and Figure 4 represent a theoretical exercise of what would happen with 100 molecules of glucose in two different environmental conditions:
Figure 2. Energy consuming effects of the Cori cycle. Left panel shows condition 1 (slightly hypoxic environment) while the center panel shows condition 2 (extreme hypoxia or anoxia). Right panel shows the effects of anoxia on the energy balance.

Figure 3. Energy consuming effects compounding in each Cori cycle. The figure shows how a further turn of the cycle increases glycolytic flux and at the same time increases energy loss.

Condition 1
This condition is characterized by an \( pO_2 \) higher than 0.5%. In this situation the oxidative phosphorylation would remain active and 30% or more glucose would be metabolized by mitochondria (Krebs cycle and electron transport chain) to \( CO_2 \) and \( H_2O \). The rest, 70% of glucose would undergo glycolysis to lactic acid. (Warburg effect: preferential glycolytic metabolism in aerobiosis). The 140 molecules of lactate thus formed are expelled to the extracellular space by the monocarboxylate transporters. Oxidative phosphorylation remains operative during the Warburg effect. In a famous debate with Warburg in 1956, Sidney Weinhouse stated that Warburg’s concept about tumor cells being unable to oxidize glucose was wrong. Furthermore, Weinhouse showed that glucose can be oxidized to \( CO_2 \) in cancer at a rate similar to normal cells. This concept has since been validated by many authors. The amount of oxidative phosphorylation that continues working after the metabolic shift varies considerably among different tumors and duration of hypoxia.

What must be kept in mind is that the Warburg effect is not the shutdown of the oxidative metabolism. It is the predominance of...
glycolytic metabolism over oxidative metabolism, but oxidative metabolism continues working. Oxidative metabolism may be decreased, equal to or greater than in normal counterparts. The presence of oxygen increases oxidative metabolism in normal and cancer cells; however, this increase is much lower in the latter. Oxidative metabolism is present even in highly glycolytic cells but operating at a lower capacity. As a conclusion, the Warburg effect is not about mitochondrial metabolism impairment (as Warburg thought) but about increased glucose uptake and glycolytic flux as postulated by Weinhouse. There is high metabolic variability among cancer types and also inside a tumor. This means that glycolytic cells may conserve variable degrees of mitochondrial metabolism.

Condition 2
In this condition, the \( pO_2 \) has decreased below 0.2%. Mitochondrial activity is practically downregulated by such a low level of oxygen. Therefore, nearly 100% of glucose is degraded to lactic acid, generating 200 molecules of lactate. Such a high lactate load can easily surpass monocarboxylate extruding capacity and a certain amount of lactate would remain inside the cell creating intracellular lactic acidosis that would endanger cancer cell survival. Activation of the Cori cycle comes to solve this situation by reconverting part of the lactate to glucose. This creates a vicious cycle in which the more glucose is degraded, the more the Cori cycle “works”, consuming four ATPs in each turn of the cycle. Each turn of the cycle increases the glycolytic flux by the generation of more glucose. This creates a vicious cycle. Figure 2 and Figure 3.

Hypoxia is an activator of gluconeogenesis (Cori cycle)
Hypobaric hypoxia has been shown to produce weight loss through diverse mechanisms including loss of appetite and activation of the Cori cycle. HIF-1\( \alpha \) and HIF-2\( \alpha \) are strongly increased with ascent above 4,000 meters of altitude. HIF-1\( \alpha \) is a transcriptional activator of phosphoenolpyruvate carboxykinase (PEPCK) which is the rate-limiting enzyme for gluconeogenesis. Experimental downregulation of HIF-2\( \alpha \) decreased gluconeogenesis in hepatoma cells (HepG2) and decreased tumor size.

The Cori cycle is a defense mechanism against lactic acidosis
Suhara et al. have shown that gluconeogenesis (Cori cycle) is a mechanism that defends against lactic acidosis. Why does the cancer cell need the Cori cycle? The need stems from the fact that the monocarboxylate transporter (MCT) system is saturable. The complete or almost complete abrogation of the mitochondrial metabolism plus the increased glycolytic flux represent such a burden that the MCT capacity is surpassed. In muscle, the half-maximal rate of lactate transport is achieved
with a lactate concentration between 13 and 40 mM\(^8\). If the maximal rate is achieved (about 20 nmol/min per μl of intracellular volume at 25°C\(^9\), the excess would remain inside the cell. Therefore, by transforming lactate into glucose or pyruvate The Cori cycle prevents intracellular lactic acidosis which would induce acidic stress and kill the cell. The velocity of lactate extrusion by MCTs is also dependent on intracellular and extracellular pH\(^{10}\). Decreased intracellular pH increases extrusion velocity, while it is lowered by extracellular acidity\(^9\). Anoxic areas of tumors have a very acidic extracellular substance, and this may slow down lactate extrusion.

The main source of glucose formed by gluconeogenesis is lactate

Koloyianni et al.\(^{83}\) and Ludholmy et al.\(^{38}\) found that in normal cells, 60% of glucose generated by gluconeogenesis used lactate as the source molecule. Glutamine and alanine contributed 10% each and glycerol 3%. The rest came from serine, glycine, and threonine.

Complex I inhibitors also inhibit gluconeogenesis

Phenformin inhibits gluconeogenesis\(^{44}\). This is a paradoxical result in the scheme, because Complex I inhibition decreases oxidative phosphorylation. However, this anti-gluconeogenesis activity of Complex I has been tested in cells that were still performing oxidative phosphorylation. It is possible that under full anaerobiosis Complex I inhibition would have no effect on gluconeogenesis.

A proof of this last concept is that pharmacological inhibition of HIF-1α reduces cancer cachexia\(^{45}\). The authors used emodin and rhein (from Rheum palmatum) to decrease HIF-1α expression. Interestingly, emodin and rhein have been shown many other anti-cancer effects\(^{46-49}\).

Mitochondrial oxidative defect produces lactic acidemia

Mitochondrial diseases that decrease mitochondrial activity can produce lactic acidemia\(^{49}\). The same happens with excessive anaerobic exercise\(^{51,92}\). However, in the case of exercise, even though the ability of MCTs to expel lactate is not exceeded, there is no intracellular lactic acidosis.

Can anoxia by itself explain the production of inflammatory and catabolic mediators?

In 1991, Ghezzi et al.\(^{81}\) showed that anoxia/hypoxia with very low levels of endotoxin was able to increase levels of TNFα, IL-1α, and IL-1β more than twofold. West et al.\(^{84}\) further confirmed these findings and added IL-6 and prostaglandin E2 to the previous list of increased cytokine production by macrophages. Macrophages were activated in an anoxic environment\(^{49}\). IL-8 production is also increased in macrophages under hypoxic conditions\(^{46}\). All these findings were confirmed by many authors\(^{40-102}\). Macrophages resistant to hypoxia modify their phenotype and achieve a high production of inflammatory mediators\(^{103}\). It is highly possible that the inflammatory mediators found in CC are the products of macrophages associated with the tumors that are subjected to the same extreme hypoxic conditions as tumors.

Can anoxia by itself explain insulin resistance?

Insulin resistance is frequently found in patients with CC\(^{104-107}\). Yoshikawa et al.\(^{107}\) found that insulin resistance in cancer patients was not caused by malnutrition.

Intermittent hypoxia induces insulin resistance\(^{108,109}\). Under normal conditions insulin is a down-regulator of gluconeogenesis. With the development of insulin resistance this, inhibition is handicapped.

Usually, tumors suffer intermittent hypoxia/anoxia rather than a permanent condition. Growth, invasion and angiogenesis create a very dynamic environment with variable conditions of oxygenation\(^{10-12}\). Furthermore, insulin resistance is a necessary development for Cori cycle activation\(^{111}\), because insulin is the main downregulator of gluconeogenesis\(^{114}\). The following circuit is probably functional in CC:

Anoxia → Glucose production → Insulin resistance

TNFα is also an inducer of insulin resistance in adipocytes\(^{115}\) and in other tissues\(^{116,117}\). TNFα is a predictor of insulin resistance in pregnancy\(^{118}\). Saghizadeh et al. found that TNFα expression was fourfold higher in the muscle of individuals with insulin resistance compared with healthy normal controls\(^{119}\). Noguchi et al.\(^{120}\) found that TNFα increased expression was associated with insulin resistance in the skeletal muscles of cancer patients. Therefore, another circuit is probably operating in CC:

Anoxia → TNFα → Insulin resistance

IL6 and IL8 also play a role in insulin resistance\(^{121}\).

Can anoxia by itself explain lipolysis?

Briançon-Marjollet et al.\(^{122}\) found that endothelin-1 (ET-1) was overexpressed in adipose tissue with intermittent hypoxia and this protein activated lipolysis.

Anoxic growth versus hypoxic growth

Since the seminal works by Semenza\(^{123-126}\), it was well established that cells grown in hypoxic medium stabilize HIF-1α, which binds HIF-1β acting as a transcription factor dimer for genes known as hypoxia responsive genes. Therefore, stable HIF-1α expression is a signal of cellular hypoxia (with the exception of those cases where HIF-1α is constitutively activated like Von Hippel Lindau disease). If, instead of hypoxia, the tumor cell is grown under anoxia, something different happens:

1) in the first 3–10 days, HIF-1α is highly expressed;
2) after the 10th day, HIF-1α is not expressed any more\(^{127}\).

In both cases inflammatory cytokines are increased many folds compared with normoxic cells. The difference between the first ten days and after that is that the cell has become fully anaerobic. The authors stated “Thus, metabolically active HeLa cells respond to the lack of oxygen, in part, by regulating...}
the levels of cytokines produced”. The increase in cytokine production was higher after 10 days of anoxia as compared with 3 days anoxia. This research clearly shows the difference in cytokines expression between short and prolonged anoxia. Figure 4 summarizes the concepts discussed above.

**Usual treatments of CC**

Many treatments have been used for CC. Not one has shown really encouraging results. Most of them address improving appetite and increasing food intake and/or supplementing calories. Other treatments target the intermediary chemokines such as tumor necrosis factor. Why all these failures? All the treatments used so far counter the symptoms and collateral effects of CC. None of them target the main (and unique) cause which is anoxia, or the main mechanism by which anoxia produces its effects, namely gluconeogenesis (Cori cycle). The failed therapies include:

- **Hydrazine sulfate**, which has been tested in cachexia treatment but has not yielded any appreciable results. However, some favorable results have been reported. “Hydrazine sulfate has shown no anticancer activity in randomized clinical trials, and data concerning its effectiveness in treating cancer-related cachexia are inconclusive”.[11]. It has not been approved by the FDA for any medical condition.

- **Steroids**. Different steroids such as medroxyprogesterone[12]-134, megestrol[135-137] have been tested for cachexia treatment. Megestrol has shown beneficial effects limited to appetite, however it does not impact cachexia and is associated with many side effects.[138]

- **Eicosapentaenoic acid**. This is an omega-3 (n-3) polyunsaturated fatty acid (PUFAs). It targets the loss of muscular mass, but does not solve the other effects of CC.[139,140]

- **High calorie nutritional supplements**.

- **High dose progestins**. These did lead to some appetite and weight improvement but without major results in the relentless evolution of CC.[141]

- **Etanercept** and **infliximab** have been used as anti-TNFα with poor results in CC[142].

- **Tocilizumab** is an anti-IL6 antibody approved by the FDA for rheumatoid arthritis treatment. It showed benefits in some cases of CC[143-145]. However, there are no randomized clinical studies confirming these benefits. The number of cases that have been published are scarce.

- **Insulin** for the treatment of insulin resistance. Lundholm et al.[146] found insulin as an important palliative treatment for patients with cancer-related weight loss.

- **Rosiglitazone** for insulin resistance treatment.

- **COX2 inhibitors like celecoxib** for decreasing acute phase pro-inflammatory cytokines. A pilot study with celecoxib showed beneficial effects in patients with CC[147].

In the next section we shall propose a treatment scheme based on targeting the physiopathology of CC, rather than the secondary symptoms.

**Discussion and hypothesis**

From a metabolic point of view, there are three types of cells in most tumors:

a) Oxidative cells located near blood vessels with adequate or near adequate oxygen and nutritional supply.

b) Aerobic glycolytic cells located in the tumor mass with inadequate oxygen supply but with a functional oxidative phosphorylation that metabolizes part of the glucose to CO$_2$ and H$_2$O; however, these cells preferentially and in major proportion use the glycolytic pathway to lactic acid.

c) Deeply anaerobic cells with near zero supply of oxygen in which only glycolysis to lactic acid is functional. These cells are unable to perform oxidative phosphorylation.

The proportion of each of these phenotypes in a tumor are variable and dynamic. At an early stage and in small tumors probably oxidative and glycolytic aerobic cells are found. As the tumor continues growing, the severely anaerobic cells, appear. The reason for this is mainly anatomic: they are in completely oxygen deprived areas. Since this last group of cells is incapable of using oxidative phosphorylation it is fully glycolytic and its lactic acid output is higher than in the other two groups.

Fully anaerobic cells have three characteristics:

a) very high level of HIF-1α expression and activation;

b) high level of intracellular lactic acid which surpasses the extrusion capacity of monocarboxylate transporters;

c) a tendency towards intracellular lactic acidosis.

HIF-1α upregulates PEPCK, activating the Cori cycle. This creates an increased energy imbalance due to a loss of four ATPs for each complete glucose-lactate-glucose cycle. In spite of the energy imbalance thus created, the new scheme rescues the cell from death due to intracellular acidification and restores the intracellular alkalinity needed for adequate proliferation.

When the proportion of severely anaerobic cells in a tumor increases, cancer cachexia develops. Usually this is the result of tumors with poor vascular supply and/or large size.

Many pro-cachexia tumors produce proteins and cytokines (whether by themselves or by stimulating other tissues) that have a lipolytic or miolytic effect such as myostatin[148]. TNFα and interferon-γ produce loss of appetite and consequently decreased food intake[149].

What is the evidence sustaining the above hypothesis?

1) The lactate shuttle is the best proof of the coexistence of oxidative cells and aerobic glycolytic cells.

2) Frequent findings of necrotic areas in large tumors prove the existence of cells that are extremely anoxic but could not implement the salvage through the Cori cycle.

3) Cancer cachexia frequently appears in the late stages of malignant progression.
4) Cancer cachexia appears progressively, as certain tumors increase in size\textsuperscript{(150)}.

The sequence of events leading to CC is shown in Figure 5 and Figure 6.

The mediator action of cytokines produced by the anoxic cells is related to loss of appetite, loss of muscle and adipose mass. HIF-1\(\alpha\) is the transcription factor that activates the Cori cycle as a salvage mechanism from the lactate overload. The center of all these activities is extreme hypoxia/anoxia/intermittent anoxia.

Figure 6 is an integrated view of the relationship between anoxia/deep hypoxia/intermittent hypoxia, on one side and the intermediaries leading to the cardinal symptoms of CC. Also the relationship among these intermediaries has been included in the drawing. This figure shows the essential link between anoxia and CC. The figure is based on the following references:

![Figure 5. Sequence of events starting in anoxia and leading to weight loss.](image1)

![Figure 6. An integrated view of the factors intervening in CC and their relation to anoxia. 1a, 2a, 3a, 4a, 5a, 6a, 7a, and 8a indicate the relation of anoxia/hypoxia on one side to the chemokines (also called toxohormones by some authors) or direct metabolic effects on the other side.](image2)
The role of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)

PGC-1α is a 798-amino-acid transcriptional coactivator considered an important activator of mitochondrial biogenesis. Interestingly, this protein has some characteristics that are important in CC:

- PGC-1α is a coactivator for the transcription of other proteins that act in energy metabolism. 
- It determines lactate metabolism.
- It activates mitochondrial fatty acid β oxidation.
- PGC-1α induces gluconeogenesis.
- It activates thermogenic genes, increasing energy expenditure.
- PGC-1α can be recruited by estrogen related receptors.
- And most importantly, it is increased in hypoxic conditions in different tissues including central nervous system.

PGC-1α, a hypoxia inducible coactivator protein increases thermogenesis and loss of energy and induces mitochondrial lipolysis. PGC-1α shows also other pro-tumoral activities in:

- Glioblastoma, where it determines a more aggressive phenotype.
- ER-negative breast tumors, in which the level of estrogen related receptors is significantly increased and probably interacting with PGC-1α. Furthermore, the association of PGC-1α with estrogen related receptors positively regulates HIF-2α transcription.
- Colorectal cancer, in which hypoxia induced overexpression of PGC-1α regulates tumorigenesis, enhancing cell motility, proliferation, stemness, resistance to chemotherapy, and reducing ROS by antioxidant enzymes activation.
- Melanoma, which when PGC-1α is overexpressed, cells show oxidative metabolism.

Our interpretation of PGC-1α over-expression in the CC is that cells try to activate the mitochondrial oxidative metabolism in a process where anoxia has near shut down this metabolic pathway. Therefore, PGC-1α is a hypoxia modulated protein that is able to control many of the intermediary steps between anoxia/hypoxia and the symptoms of CC.

Treatment proposal

In this paper we argue that anoxia is the main cause of CC. Figure 5 and Figure 6 shows how anoxia achieves all the cardinal symptoms of CC through diverse mechanisms. The figure does not show the intracellular lactic acidosis, because the Cori cycle prevents its onset.

Based on Figure 5 and Figure 6 we must first target anoxia and the Cori cycle. Then we can attack the symptoms. Going against the symptoms alone has failed consistently in the past.

The scheme proposed here:

- Anoxia is difficult to target. Vasodilators such as nitrates and “hemodynamic improvers” such as pentoxifylline may improve circulation in the tumor decreasing anoxia. Plunarizine is another vasodilator that has been tested in tumors. A combination of these drugs should enhance tumor oxygenation. Any anti-angiogenic drugs being used, should be discontinued.
- HIF-1α, directly upregulated by anoxia, is a targetable transcription factor. Although extensive and intensive research for an inhibitor has been going on for many years, no adequate drug has been developed yet. Emodin is a non-toxic inhibitor of HIF and may produce some benefits in CC.
- Intracellular lactic acidosis-Cori cycle-gluconeogenesis: one of the mechanisms to decrease Cori cycle is to impede excessive lactic acid production, whether in the anaerobic cancer cells or in the liver. In this sense probably the drug of choice should be dichloroacetate (DCA).
- Improving appetite: if the previous issues have been medicated and controlled to a certain extent, then improving appetite with megestrol and administering high calorie nutritional supplements makes sense.

The fundamentals for the treatment scheme

**Metabolic modifier: DCA**

DCA is an investigational drug for lactic acidosis, pulmonary hypertension and now for cancer. In some African countries, its use is unofficially accepted for the treatment of lactic acidosis in children with malaria. It is a small molecule with the following formula HOOC-CH₂-Cl. DCA is an orally available molecule that is almost completely and quickly absorbed at the digestive system. It is metabolized by GSTZ1 (a glutathione transferase isofrom). When DCA was administered to 16 healthy individuals, in 1 to 50 mg/kg IV infusions, it lowered plasma glucose, lactate and alanine concentrations. Plasma levels linearly followed those of the administered dose up to 30 mg/kg. A dose of 35 mg/kg was considered most effective regarding lactic acid, which fell to 75% below baseline concentrations within 2 hours of the infusion. Blood glucose was not affected in these healthy individuals but was reduced in diabetic patients through
stimulation of peripheral glucose utilization and inhibition of gluconeogenesis. DCA also inhibits lipogenesis and cholesterol synthesis. Daily oral administration of 50 mg/kg DCA to diabetic patients with slightly elevated lactic acid concentration, reduced alanine and lactic acid in plasma.\(^{241}\)

The experimental administration of a single dose of DCA may be misleading upon dose and metabolism, because as Gonzalez-Leon et al.\(^{242}\) have shown in rats with repeated administration of DCA, this drug has a surprising feature: it inhibits its own metabolism. This means that the chronic administration of DCA differs from single doses in its plasma concentration, toxicology and metabolism. Figure 7.

In humans, the maximum plasma concentration achieved with an IV infusion of 10 mg/kg was between 19.9 μg/ml and 24.7 μg/ml with a half life of only 20 minutes. When the infused dose was increased to 20 mg/kg the plasma concentration was between 57.3 and 74.9 μg/ml with a half life of 36 minutes. In dogs and rats the half life was much longer (the half life of a 100 mg/kg dose was 4 hours and 24 hours in dogs and rats respectively\(^{243}\)). These marked differences among species cast doubts about the possibility of translating the findings in other mammals to humans.

Confirming these inter-species differences, Maissenbacher et al. have found that dogs present enhanced inhibition of DCA degradation and slower clearance than humans and rats due to increased inhibition of GSTZ1\(^{244}\). The DCA metabolic pathway starts with dehalogenation to monochloroacetate and glyoxalate and then it continues to glycine, and the final products are oxalate and carbon dioxide\(^{245}\). The first dose is cleared from plasma faster than subsequent doses, as Gonzalez-Leon et al. have shown; this is probably due to GSTZ1 inhibition by DCA in subsequent doses. This is similar in all species. The decrease in DCA clearance by multiple doses is not a minor issue, because the initial clearance may be reduced to less than 25% of the initial one in successive doses\(^{246}\).

The main mechanism of action of DCA is the inhibition of pyruvate dehydrogenase kinase (PDK) and its isofoms. This inhibition increases the flux of pyruvate into the mitochondria, promoting glucose oxidation instead of glycolysis.\(^{247}\) PDK inactivates the pyruvate dehydrogenase enzyme complex through phosphorylation. By downregulating the activity of this complex, PDK decreases the oxidation of pyruvate in mitochondria and increases the conversion of pyruvate to lactate in the cytosol.

Other mechanisms of action of DCA in cancer can also be found in the medical literature. Stockwin et al.\(^{248}\) described that cytotoxicity of DCA is only achieved in those cells that suffered mitochondrial DNA mutations that “condemn” them exclusively to the glycolytic pathway. Therefore, DCA has features that make presume it will reduce glycolysis, lactic acid production and gluconeogenesis in anaerobic malignant cells or even cause their death.

Why DCA?

- TNF\(\alpha\) and IL-1\(\alpha\) are inhibitors of pyruvate dehydrogenase and mitochondrial metabolism\(^{249,250}\). DCA has exactly the contrarian effect.
- DCA decreases the expression of TNF\(\alpha\) and IL-1\(\beta\) and lactate production in ischemic insults\(^{251}\). It also decreases the expression of IL-6 and Interferon \(\gamma\)\(^{252}\).
- While TNF\(\alpha\) increases fermentative glycolysis, DCA decreases it\(^{253}\).
- DCA is an inhibitor of lipolysis\(^{254}\).
- DCA decreases all the gluconeogenic precursor molecules\(^{255}\) and thus probably decreasing gluconeogenesis.

![Figure 7. Differences in DCA clearance between first and subsequent administration. This is an important issue to be considered for the clinical use of DCA.](image-url)
• DCA seems to reduce insulin resistance\textsuperscript{256}.

• It has been suggested that DCA had the ability to decrease/block the Cori cycle\textsuperscript{257,258}.

• DCA showed inhibitory effects on HIF-1\(\alpha\) in glioblastoma cells\textsuperscript{259,260}.

• DCA synergizes with other chemotherapeutic drugs\textsuperscript{261–266}.

• DCA targets mainly cells that cannot use the oxidative metabolism\textsuperscript{281}. This concept is further confirmed by the synergy between DCA and metformin\textsuperscript{267–269}. The fundamentals of this association stem from the fact that metformin inhibits mitochondrial Complex I and reduces oxidative metabolism while DCA inhibits glycolysis. This double-edged approach would target very hypoxic cells where oxidative metabolism is minimal and is even further blocked by metformin.

• According to the effects discussed above, DCA seems the only drug that targets simultaneously most of the proteins and pathways involved in CC pathogenesis.

**Increasing tumor oxygenation and inhibiting TNF\(\alpha\): Pentoxifylline, nitroglycerin and thalidomide**

**Pentoxifylline** reduces the expression of TNF\(\alpha\) in cancer cells. It has been tested in CC in five patients with good results in three of them\textsuperscript{270}. Many other reports have confirmed the inhibitory actions of pentoxifylline on TNF\(\alpha\)\textsuperscript{271–274}, not only in cancer, but also in other pathologies\textsuperscript{280,281}. Pentoxifylline has other very important actions in CC: it is a hemorheological agent that increases red blood cell deformability, reduces blood viscosity and decreases platelet aggregation improving microcirculation\textsuperscript{282}. Therefore, a better oxygenation of the tumor is expectable. However, a study by Goldberg et al.\textsuperscript{283} with pentoxifylline as a stand-alone drug did not show any improvement in patients with CC. There is evidence that pentoxifylline increases tumor oxygenation\textsuperscript{284,285}.

**Nitroglycerin** (NTG) is another drug that deserves consideration. It is a well-known vasodilator and oxygenation improver. In addition to these effects NTG also reduces HIF-1\(\alpha\) levels in hypoxic tumors\textsuperscript{266,267}, because it acts as a nitrous oxide donor. Unfortunately, NTG has both pro and anti-tumor effects\textsuperscript{286}. However, used on a short-term basis as an adjunct to pentoxifylline, an important improvement of tumor oxygenation can be expected. For a review of nitroglycerin’s anti-tumoral activity, read Sukhatme et al.\textsuperscript{287}.

**Thalidomide** is a TNF\(\alpha\) downregulator\textsuperscript{288} and has been tested in CC with encouraging perspectives\textsuperscript{289}. It had similar results in wasting syndromes of other origins\textsuperscript{290,291}. Thalidomide has also other anti-cancer effects\textsuperscript{292}, such as anti-angiogenesis and T-cell stimulation\textsuperscript{293}, which will not be considered here because they go beyond the scope of this manuscript. It has been established as part of multiple myeloma treatment protocols. For a review of thalidomide, read Luzzio\textsuperscript{294}.

**Downregulation/inhibition of HIF-1\(\alpha\)**

As mentioned above, emodin can be used for this purpose. Emodin down-regulates HIF-1\(\alpha\) expression\textsuperscript{295}. It also inhibits pro-inflammatory responses\textsuperscript{296} that play a role in CC. Furthermore, hepatic cancer cells (HepG2) treated with emodin showed a significant decrease of lactate. This decrease is a signal of glycolytic inhibition which has been further confirmed because emodin decreased mRNA levels of hexokinase II (HKII), pyruvate kinase isomor M2 (PKM2), and lactate dehydrogenase-A (LDHA) in a concentration-dependent manner\textsuperscript{297}. Emodin also inhibits TNF\(\alpha\), NF-\(\kappa\)B and IL-6, all mediators of CC\textsuperscript{298}. Emodin has many other anti-cancer effects, such as:

• sensitization to chemotherapeutics\textsuperscript{299–303},

• increasing ROS production\textsuperscript{304},

• promoting apoptosis,

• inhibiting angiogenesis\textsuperscript{305,306}, metastasis\textsuperscript{307}, migration and invasion\textsuperscript{308},

• inducing proteasomal degradation of Her2/neu\textsuperscript{309},

• inhibiting ATP citrate lyase\textsuperscript{310},

• increasing expression of insulin-like growth factor binding protein-1\textsuperscript{311},

• reverting cisplatin resistance\textsuperscript{312},

• blocking STAT 3 activation\textsuperscript{313}, among others.

For a review on emodin pharmacology see Dong et al.\textsuperscript{314}.

The treatment proposed here includes the association of pentoxifylline, emodin and DCA as an added scheme to classical treatments such as high calorie nutritional supplements and anabolics like megestrol (Figure 8). Adding these drugs to the conventional treatment would decrease anoxia, HIF-1\(\alpha\) expression, and the glycolytic pathway restoring oxidative phosphorylation and reducing TNF\(\alpha\) expression. This type of treatments targets the etiology of CC rather than the symptoms.

**Inhibition of PEPCK to block gluconeogenesis**

PEPCK is the rate-limiting enzyme for gluconeogenesis. Therefore, its inhibition should block the Cori cycle. Many drugs have been identified with the ability to inhibit PEPCK, such as metformin\textsuperscript{315}, troglitazone\textsuperscript{316}, berberine\textsuperscript{317}, among others. None of these drugs have been tested in CC. Berberine should be considered a particularly interesting drug because it inhibits PEPCK but also downregulates HIF-1\(\alpha\)\textsuperscript{318}. Berberine also has many other anti-cancer effects\textsuperscript{319}, such as down-regulation of COX2\textsuperscript{320}, increased apoptosis in cancer cells without affecting
the normal ones, reduced migration and invasion, among others. For a review of other anti-cancer effects of berberine, read Kaboli et al.

Other potential drugs
3-bromopyruvate (3BP) is a protein alkylating agent that has shown many anti-cancer effects. We included it in this list of possible drugs for treating CC because it is a potent inhibitor of aerobic glycolysis. Inhibition of glycolysis should decrease lactate production, thus decreasing the Cori cycle. The exact mechanism of action is not fully known, but there is some evidence pointing to inhibition of glycolytic enzymes. It has important cytotoxic effects on highly glycolytic tumor cells.

Tocilizumab (a humanized anti-IL6 receptor antibody) may produce some benefits. It requires further testing. The association of tocilizumab with gemcitabine for the treatment of advanced pancreatic cancer failed to show clear clinical benefit in a phase I/II clinical Trial.

Insulin, besides its known actions (inhibitor of gluconeogenesis), exerts inhibitory activity on PGC1α expression.

Anamorelin is a small molecule ghrelin receptor agonist that has shown favorable effects on appetite, food intake and weight gain in patients with CC. However, its approval was rejected twice by the European Medicines Agency.

An alternative hypothesis: the browning of adipose tissue
Petruzzelli et al. reported a completely different physiopathological road leading from cancer to cachexia: the browning of white adipose tissue. They maintained that a phenotypic switch from white adipose tissue to brown adipose tissue metabolism was the main culprit of CC. The main characteristic of browning would be the increased expression of uncoupling protein-1 in white adipose tissue, with consequent high energy expenditure. They also found that inflammatory intermediaries (mainly IL-6) were the cause of the browning process and proposed the anti-inflammatory sulindac for CC treatment. Considering that the findings of Petruzzelli et al. are correct, one question remains unanswered: why do advanced tumors produce significant inflammatory mediators? And this takes as back to the anoxia problem: it is anoxia that induces the production of inflammatory mediators.

The steps between anoxia and CC can be those proposed by Petruzzelli et al. (browning of white adipose tissue) or those hypothesized in this paper (increased Cori cycle). The increased Cori cycle can occur in the tumor, in the liver or in both. However, the primum movens remains anoxia. Treating the intermediate steps (TNFα, IL-6 or other chemokines), or the symptoms (loss of weight, lipolysis, muscle loss) are valid approaches. However, the only significant result would be achieved by simultaneously targeting fermentative glycolysis and anoxia alongside to the other treatments.

A unified explanation of the causes of CC has not been achieved yet. Therefore, anoxia as the unifying cause behind CC deserves more research.

Conclusions
A unitary explanation of the cause of CC is presented here. The main culprit of this wasting syndrome is anoxia. The molecular mechanisms leading from anoxia to the full blown syndrome are
also presented. A therapeutic approach, based on this hypothesis is proposed.

Anoxia in large areas of the tumor mass is the main cause of CC. This occurs through a sequence of events where oxidative phosphorylation is almost totally shut down leading to full glycolytic behavior (100% of the glucose is degraded through fermentation and none through oxidation). Vascular supply and cell metabolism are highly heterogeneous throughout the tumor. Anoxic anaerobic metabolism is also present in parts of the tumor mass. When an important portion of the tumor is “pushed” to fully anaerobic metabolism by lack of oxygen, CC develops. Even the most recent publications on CC miss the central issue: anoxia. Therefore, there is no place for anti-oxi-anoxic treatments in the therapeutic protocols being used routinely. Anoxia produces such a high level of intracellular lactate that it surpasses monocarboxylate transporters extruder capacities. Thus, the Cori cycle is triggered to prevent intracellular lactic acidosis creating an energetic imbalance due to the cycle’s high energy requirements. Increased inflammatory mediators, that cause many of the symptoms of CC, are not produced by the tumor itself but by hypoxia resistant macrophages associated to the malignant stroma.

All the treatments employed up to now have failed because they addressed the symptoms of CC instead of the causes. Here we propose targeting anoxia, HIF-1α, and the glycolytic pathway as the logical treatment for CC using a combination of drugs such as pentoxifylline, dichloroacetate, metformin and emodin associated with anabolic steroids and nutritional supplements. The drugs can be changed for others with a similar effect. What is important is to center the treatment on tumor anoxia, the glycolytic pathway and TNF. It is probably useless to address only one of these issues or expect real improvements with nutritional supplements and appetite improvers. The usually late onset of CC in a prolonged disease and the frequent therapeutic failures have paved the way for a nihilistic attitude that has prevailed up to the present. Targeting the strongly anaerobic cells in the tumor will not only improve CC but at the same time slow down the disease and eventually prolong survival. DCA and the association of DCA with metformin, vasodilators, and HIF-1α inhibitors deserve well planed experimental and clinical research for CC’s therapy.

Data availability
No data are associated with this article.

References


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