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Research Article

Nuliglucaemia Lucidae: Extreme Deprivation of Blood Glucose as Organic Context for Cancer Treatment with Antimetabolites

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Abstract

The present report describes our clinical findings regarding the use of high dose intravenous insulin in cancer patients, as a means to deplete the blood compartment of glucose molecules. The purpose of this intervention is to create a favorable physiological state for the competitive inhibition of several rate-limiting enzymes within cancer cells, with structural analogues that behave as antimetabolites. Regardless of their histological origin, virtually all solid tumors reported on to date (February 2020) are found to be hypercaptant in PET-CT scans following the intravenous injection of 2-^{18}fluoro-deoxy-D-glucose. Most solid tumors display a hypermetabolic phenotype (SUVmax ≥ 3), with marked overexpression of glucose transporters (GLUTs) in the outer membrane of their anaplastic cells subpopulations. The fact that neoplastic cells also overexpress glycolytic, fermentative and glutaminolytic enzymes up to an order of magnitude relative to healthy cells further strengthens the argument for a competitive inhibition with antimetabolites. The rationale for a deep, systemic deprivation of glucose was suggested by classical enzymological work concerning competitive inhibition (the Woods principle), and our group has shown that a metabolic intervention with structural analogues of glucose and pyruvate is strongly enhanced by a systemic suppression of the natural substrates of hexokinase 2 (HK-2) and lactic dehydrogenase isozyme A (LDH-A), followed by the timely introduction of several non- metabolizable analogues. Sustained, deep hypoglycemia (<10mg/dl) under physiological ketosis provides an advantageous context for antitumor treatment with structural analogues of glucose, pyruvate and glutamine. Data provided in this report demonstrates the feasibility and safety of the procedure.

Keywords: physiological ketosis; insulin clamp; competitive inhibition; metabolic cancer therapy

Preliminary considerations

A favorable metabolic context for pharmacological interventions against cancer with glucose and glutamine analogues has been devised by our clinical research group [1]. This system has been safely and successfully implemented *in Homo* for the last twelve years in our Centro de Terapia Metabólica. We have forwarded the term "*nuliglucaemia*" to designate a state in which circulating blood glucose becomes virtually undetectable by standard hand-held instruments, dropping under 18 mg/dl and even to single-digit levels; whereas the adjunct term "*lucidae*" describes the fact that patients remain conscious, *i.e.* lucid, being able to answer simple questions (name, personal address, social security number), with no long-lasting adverse effects on their brain function. The realization that such a state can be achieved safely in the clinical setting is in stark contrast with commonly held beliefs within the field.

Considerable clinical experience already reported by these authors has proven that such an energy blockade of tumoral metabolism is indeed possible, without damaging the organism of the host [2]. The aim of this intervention is the onset of an acute energy disturbance in neoplastic tissues, sparing healthy organs. Such an approach is an exploitation of the functional asymmetry between neoplastic cells and healthy neighboring cells with a therapeutic aim. Evidence of such functional asymmetry is readily apparent in positron emission tomography studies (PET-CT), and references to cancer hypermetabolic phenotype are also abundant in the literature [3-6]. Robust *in vitro* studies have shown the respiratory quotient (RQ=CO2/O2) of ascites cells and isolated neoplastic cells to be invariably depressed even in the presence of sufficient paO2 in the culture medium, *i.e.* the Warburg effect [7-9]. Recently, evidence of ultrastructural pathology in human astrocytomas has been obtained by transmission electron microscopy, providing visual confirmation for Warburg's seminal findings on the facultative anaerobiosis of neoplastic cells, and his observation that cell respiration is "structure bound", *i.e.* intact inner mitochondrial cristae are a material requirement for oxidative phosphorylation [10-12]. Concerning ultrastructural abnormalities, our group has suggested the term *crestodysmorphia* to describe this peculiar morphological feature of cancer cells mitochondria. From the clinical perspective of competitive inhibition of the rate-limiting enzymes of solid tumors, it is semiologically relevant that neoplastic tissues show such a strong avidity for glucose, as ascertained semi-quantitatively by SUVmax calculations [Mbq Region of Interest/(Mbq injected/lean body mass)]. In this regard, PET-CT has provided proof-of-concept for a metabolic approach to cancer treatment. For diagnostic purposes, the cut-off has been conventionally set at ≥ 3 and reported measurements have been as high as 73 [13]. This last measurement implies a signal-to-noise ratio of

1 to 73, meaning the Region of Interest has a seventy-three fold increased avidity for glucose relative to surrounding healthy tissues.

The issue of brain fuel

In the medical lore, there is a deeply rooted belief that neurons can feed exclusively on glucose. Glycemic levels under 70 mg/dL are regarded, therefore, very dangerous and are vigorously corrected [14]. It seems to be common knowledge that blood glucose levels below 50 mg/dl "immediately trigger seizures", with subsequent "irreversible brain damage" and so forth. However, central nervous system cells can perfectly extract energy through the oxidation of ketone bodies β-hydroxybutyrate ($C_4H_7O_3^-$) and acetoacetate ($C_4H_5O_3^-$), which serve as an alternative fuel during fasting and, conspicuously, even under a thorough removal of blood glucose [15-17]. Ketone bodies have even been shown to suppress glucose consumption by brain cells [18, 19]. The essential, in fact, the *sine qua non*, condition to make this clinical technique tolerable, is that, in the date of the procedure, the subject arrives in a state of *physiological* ketosis, having made the necessary transition or metabolic shift to a low glucose/high ketone state by means of caloric restriction, with fatty acids beta-oxidation already occurring in a steady state. Inadequate preparation could indeed result in a critical loss of *all* brain fuel, leading to seizures and neuronal damage [20, 21]. Having performed over twenty thousand such intravenous insulin clamps over the course of eleven years, on two thousand plus tumor-bearing patients, we recognize this procedure as feasible and safe. Furthermore, under proper conditions, even adverse reactions -should they occur- could easily be corrected within seconds by intravenous injection of 5cc of a standard hypertonic glucose solution. Further corrections could be made if needs be.

Materials and methods

Eighty-five patients, 47 females 38 males, were selected to evaluate their Critical Response Insulin Sensitivity (CRIS) index. Ages ranged from 35 to 72 years ($\bar{\mathbf{x}}$= 53.6), all with a confirmed diagnosis of cancer, and a BMI ≥19, therefore susceptible to dietary restrictions. For the purpose of determining the effective personal dose (EPD) of insulin to be administered during each treatment session under the CISA protocol, a previous exploratory test was conducted to assess each patient overall organic status and insulin sensitivity, as well as their degree of compliance with the dietary restrictions necessary to enter a state of *physiological* ketosis. The data presented herein result from a subsequent, definitive test, administering the calculated full megadose of fast delivery insulin. Physiological ketosis, distinctly different from pathological ketoacidosis and hyperglycemic hyperosmolar syndrome (HHS) of decompensated diabetic patients, was defined as a ratio of ketone/glucose ≥0.03. Plasma pH in all 85 samples was 7.4 (± 0.1). Ketone bodies were measured with a commercial hand-held device, FreeStyle (Abbot). Peripheral blood samples for glucose measurements were collected in NaF tubes (0.86 mol/l). Quantitative glucose determination was performed by the enzymatic-Uv method using hexokinase, on the A15 Clinical Chemistry Analyzer (Biosystems).

The procedure routinely starts with the evaluation of the patient's condition and quantification of physiological ketosis by the operator. Following catheterization of either the cephalic or basilic vein, a three-way stopcock with a Luer lock (Discofix) is attached to the Jelco catheter (Smith Medical), connecting an IV bag to the distal port. Once the intravenous route has been secured and kept permeable by a continuous drip (7 drops per minute) of isotonic saline solution 0.9% (B BRAUN) the operator proceeds to administer the corresponding dose of insulin (Lilly) -Humulin R, 100 IU x millilitre- in one single bolus injection. Within this group, the insulin EPD ranged from 20 IU to 70 IU ($\bar{\mathbf{x}}$= 23, $\tilde{\mathrm{X}}$ = 25).

Close and constant attention was paid to developing signs and symptoms throughout the test by trained medical personnel. Measurements were taken at 7-minute intervals, carefully registering every discernible and/or referred changes in the patient's state. Blood specimens were sent to our laboratory at a consistent pace, under the direct supervision of the technical director/chief technician in order to minimize preanalytical errors.

Recovery was induced through the ingestion of medium chain triglycerides and other fats, by means of a high fat/normal protein broth provided (drank at ≈ 60°C). Upon spontaneous normalization of blood glucose, once they regained total motor coordination and mental acuity and were perfectly capable of managing themselves unaided, patients were released to return home.

Before each individual test, written informed consent was obtained, and both patients and their close relatives were previously instructed in every instance on the necessary preparations and precautions.

On instrumental limitations and degree of uncertainty

The measurements of blood glucose we are reporting on were performed through the hexokinase method (margin of error ± 0,02 mg/dl), a transferase involved in the catalysis of hexoses by means of a reaction with adenosine triphosphate [22]. The low Km for hexokinase (10^{-5}M or 0.9 mg/ %), allows it to operate at V_{max} even at sub-physiological glucose concentrations, such as those expected during our tests. The quantitative measurement of glucose by the hexokinase-UV method, based on original work by Schmidt *et al.* and subsequently reproduced by Peterson and Young [23, 24] a long-established reference method based on the principle of exclusive substrate-enzyme interaction, provides higher specificity and sensitivity relative to conventional methods of quantitative glucose determination (GOD/PAP). For the measurement of blood ketones, commercial hand-held device FreeStyle (ABBOT), which has proven to perform consistently across many weeks of heavy use, was found to have a delta of 0.07 mg/dl.

Results

Baseline beta-hydroxybutyrate levels at the beginning of the test ranged from 0,8 mmol/l to 8 mmol/l ($\bar{\mathbf{x}}$ = 4.9), while baseline blood glucose levels ranged from 38 mg/dL (1.8 mmol/) to 98 mg/dL (5.4 mmol/l) ($\bar{\mathbf{x}}$= 68.9 mg/dl or 3.8 mmol/l). Tested at 7-minute intervals, subjects registered 3 to 11 measurements at the single-digit region (≤ 9 mg/dl, or 0.5 mmol/l). A minimum of 16 intervals was registered, with 85 patients remaining in the *nuliglucaemia lucidae* state (therefore, by definition, under the 18 mg/dl Abbot Glucometer threshold) for more than 9 intervals, or 63 minutes (Figure 1). The lowest glucose level registered was 2 mg/dl (0.1 mmol/l) (Figure 2). All patients remained lucid during the test, no adverse effects were registered.

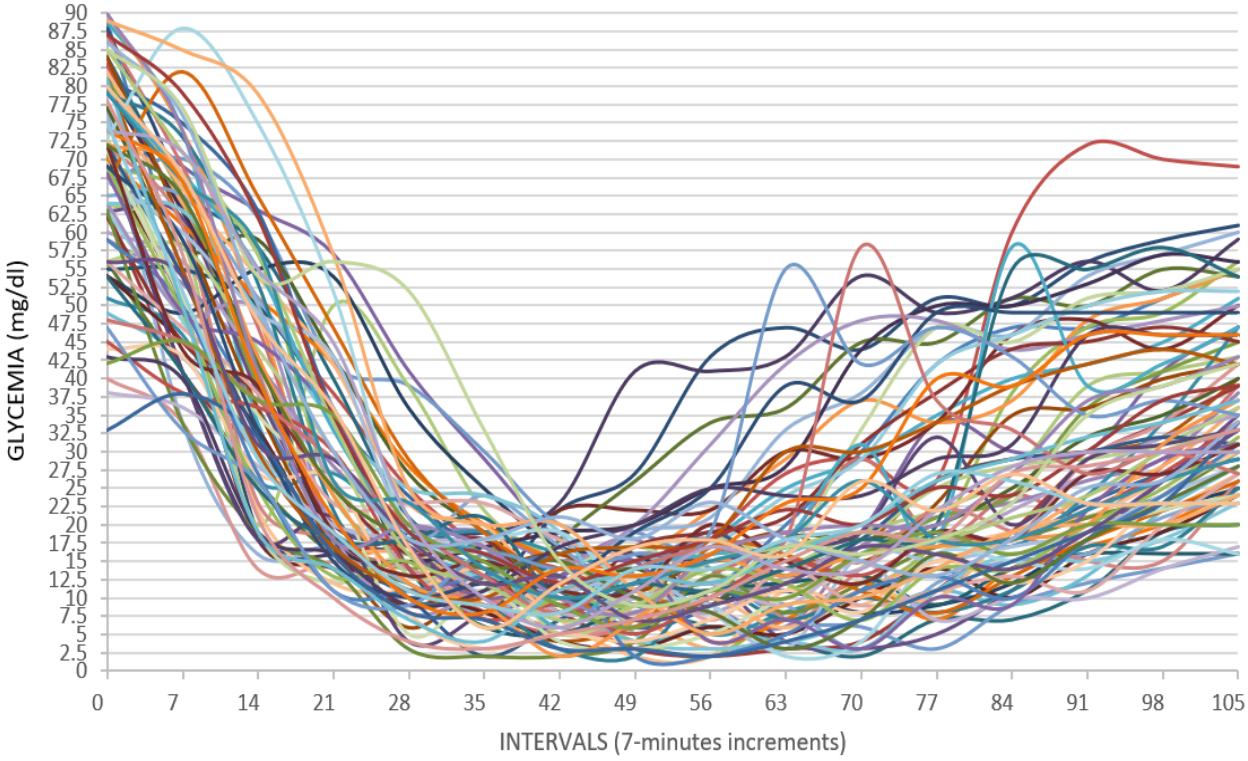

Figure 1: Full blood glucose measurements for each subject (N=85) throughout 16 intervals.

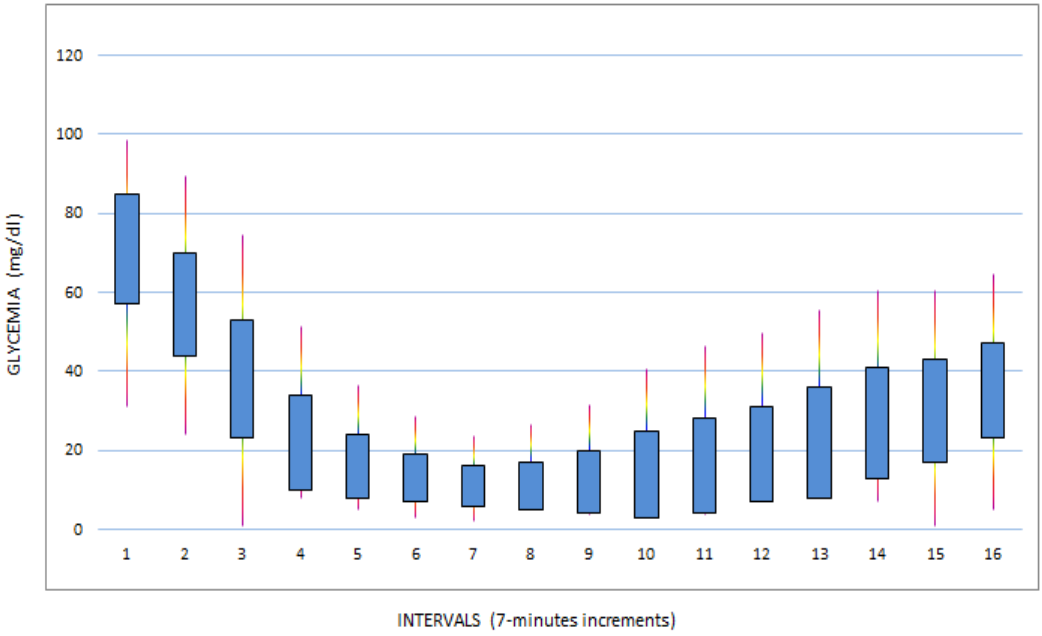

Figure 2. "Japanese" candlesticks graph of glycemic measurements for all subjects. Glycemic range at each interval (all measurements registered).

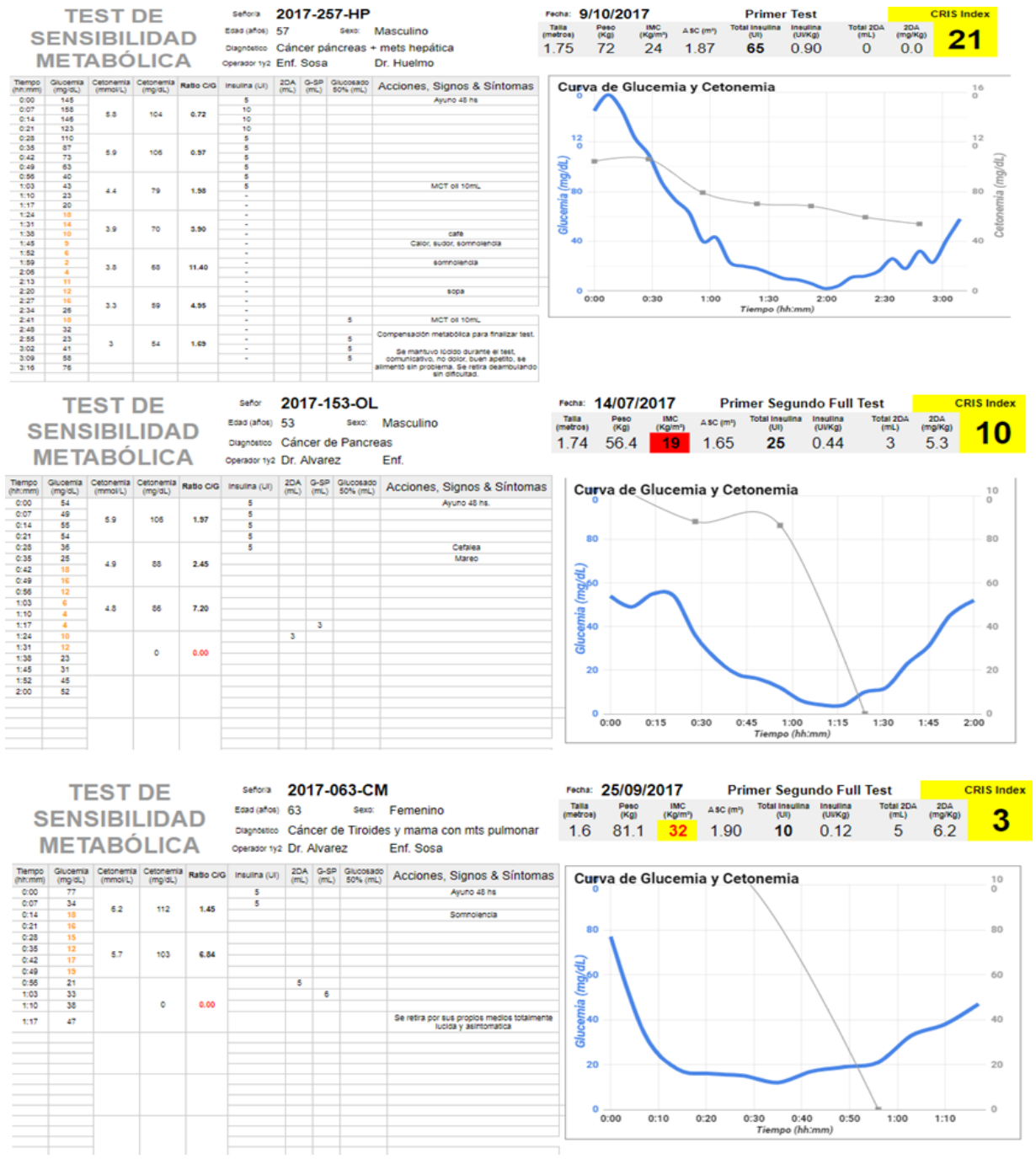

Figure 3. Three examples of individual test blood samples, with detailed notations. The charts display insulinemia levels following megadose insulin -bolus injection- in a subset of 3 patients (random selection). Blood glucose levels were measured both through the Glucose Oxidase method (Abbot) -with inherent instrumental limitations- and the Hexokinase method (Biosystems). Highlighted in yellow, on the upper right corner of each chart, is the Critical Response Insulin Sensitivity (CRIS) index. The seven-fold variation in the CRIS index between patients 063-CM and 257-HP is not uncommon and is a strong predictor of treatment outcome.

On the Critical Response Insulin Sensitivity index

The Critical Response Insulin Sensitivity (CRIS) index (Individual test tables 1-3, upper right box), is a quantitative measure of the patient's degree of insulin resistance assessed under conditions of deep physiological stress. Though similar to the alarm phase of the General Adaptation Syndrome first described by Selye *et al*. [25, 26], in this instance the critical response is not triggered by external stimuli but brought about by a megadose insulin challenge. Relative to the Oral Glucose Tolerance Test (OGTT), stress-testing the resistance of the host by a megadose intravenous insulin challenge truly explores the opposite end of the continuum of mammalian metabolism. This has proven to be of clinical relevance in tackling the cancer conundrum, characterized by hypermetabolic behavior. The standard OGTT explores the *reverse to the mean* from a state of glucose over-abundance, extrapolating data thus

obtained to qualitatively assess the insulin sensitivity of the host. The CRIS index, conversely, uncovers much deeper aspects of the host physiology, thus helping in the design of a personalized, precise metabolic intervention. Interindividual differences of the CRIS index among tumour-bearing patients can vary by an order of magnitude. Throughout both instances -overabundance and total lack of glucose- several homeostatic mechanisms attempt a *reverse to the mean*, but the hormonal and biochemical responses involved are strikingly different. It is in the midst of the systemic perturbation brought about by such acute hormonal disruption that several physiological phenomena -not previously explored in regards to cancer treatment- can be taken advantage of. The calculation of the CRIS index is carried out by the following formula:

(IU of EPD/ Kg BWT patient)

(IU of EPD/ Kg BWT ideal subject)

where EPD = Effective Personal Dose -in International Units of potency-, the amount of insulin per kilogram of body weight (BWT) that brings about at least 4, but no more than 12, *sequential* blood glucose measurements <18 mg/dl (1 mmol/l). Whereby the number arrived at is a unitless quantity describing the insulin resistance of the host as a multiple of an ideal subject's resistance. This was a working concept arrived at by testing a series of young, lean, 70 kg (± 0.6) athletes with a single intravenous injection of 3 IU of insulin. Testing these metabolically ideal individuals gave a mean EPD/Kg of .04 IU (data not shown), subsequently used as a yardstick to quantify insulin resistance in tumor-bearing patients.

Limitations of the study

Regrettably, these techniques offer no insight into the glutamine metabolism of the host. Given the complexity of tumor metabolism and the fact that glutamine has been found to be an important nutrient for neoplastic cells [27, 28] a parallel approach should be devised for clinicians to simultaneously assess glutaminemia, as well as the rate of glutamine catalysis into glutamate and alpha ketoglutarate within neoplastic tissues.

Conclusion

Absolute deprivation of blood glucose is achievable in the clinical setting at virtually no risk, provided protocol is strictly followed. For the purpose of enzymatic inhibition with antimetabolites of glucose, pyruvate and glutamine, a particular subpopulation of cancer patients with a BMI above 19, and under the age of 70 years could safely be induced in a state of *nuliglucaemia lucidae.* Intravenous, megadose, insulin-induced hypoglycemia provides a material advantage to the treatment of solid tumors by means of competitive inhibition with structural analogues. Incidental findings regarding a high correlation between the proprietor indicator CRIS index and survival has led us to believe that a population-wide screening tool for quantifying metabolic status could -and therefore should- be devised to allow for truly early detection of neoplastic disease.

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