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Warburg effect and Intralipid effect on the mitochondria of cancer cells

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Abstract
Otto Warburg observed that cancers ferment glucose in the presence of oxygen, suggesting that defects in mitochondrial respiration may be the underlying cause of cancer.
As stated by Otto Warburg nearly a century ago, cancer is a metabolic disease, a fermentation caused by malfunctioning mitochondria, resulting in increased anabolism and decreased catabolism. Treatment should, therefore, aim at restoring the energy yield.
It was shown that a lipidic emulsion (LDE) composed of phospholipids and cholesterol esters which binds to low-density lipoprotein (LDL) receptors may concentrate in acute myeloid leukemia cells.
LDE concentrates much more in malignant breast tumor tissue than in the normal tissue.
Key words: *Warburg effect, Mitochondria, Intralipid. Lipid Emulsion.*

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Dr. Otto heinrich warburg

Otto Heinrich Warburg (1883-1970) was a member of an illustrious Jewish family, known for some five centuries. From humble beginnings, the family became prominent in the world for their contributions to all aspects of society. The son of a German mother and a Jewish (converted) father, Otto H. Warburg became a major contributor to medical science in the field of cancer research. Considered for Nobel Prize more than once, he finally received it in 1931 for his discovery of the nature and mode of action of the cellular respiratory enzyme. Warburg's personality was controversial: he was intolerant of opposing scientific views yet tolerant toward Nazi abuses. Accused of collaboration under the Nazi regime, Otto H. Warburg was nevertheless readmitted to the global scientific community after World War II. His contribution to cancer research remains influential to this day and has been superseded by discoveries that have built upon his work (1).

Decades ago, Otto Warburg observed that cancers ferment glucose in the presence of oxygen, suggesting that defects in mitochondrial respiration may be the underlying cause of cancer. We now know that the genetic events that drive aberrant cancer cell proliferation also alter biochemical metabolism, including promoting aerobic glycolysis, but do not typically impair mitochondrial function. Mitochondria supply energy; provide building blocks for new cells; and control redox homeostasis, oncogenic signaling, innate immunity, and apoptosis. Indeed, mitochondrial biogenesis and quality control are often upregulated in cancers. While some cancers have mutations in nuclear-encoded mitochondrial tricarboxylic acid (TCA) cycle enzymes that produce oncogenic metabolites, there is negative selection for pathogenic mitochondrial genome mutations. Eliminating mtDNA limits tumorigenesis, and rare human tumors with mutant mitochondrial genomes are relatively benign. Thus, mitochondria play a central and multifunctional role in malignant tumor progression, and targeting mitochondria provides therapeutic opportunities (2).

Cancer cells consume more glucose by glycolytic fermentation to lactate than by respiration, a characteristic known as the Warburg effect. In contrast with the 34 moles of ATP produced by respiration, fermentation produces two moles of ATP per mole of glucose consumed, which poses a puzzle on the function of the Warburg effect. Productions of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) per mole linearly vary with the fraction (x) of glucose consumed by fermentation that is frequently estimated around 0.9. Hence, calculation shows that, in respect to pure respiration, the predominant fermentative metabolism decreases around 10% the production of entropy per mole of glucose consumed in cancer cells. We hypothesize that increased fermentation could allow cancer cells to accomplish the Prigogine theorem of the trend to minimize the rate of production of entropy. According the theorem, open cellular systems near the steady state could evolve to minimize the rates of entropy production that may be reached by modified replicating cells producing entropy at low rate. Remarkably, at CO2 concentrations above 930 ppm, glucose respiration produces less entropy than fermentation, which suggests experimental tests to validate the hypothesis of minimization of the rate of entropy production through the Warburg effect (3).

Mitochondria in cancer cells

Prominent features of cancer cells include metabolic imbalances and enhanced resistance to mitochondrial apoptosis. The fact that tumors rely heavily on glycolysis to meet their metabolic demands has been recognized since the beginning of the twentieth century, yet a complete elucidation of the so-called Warburg effect has not been achieved. Several mechanisms have been proposed to explain this phenomenon, including the upregulation of rate-limiting steps of glycolysis, the accumulation of mutations in the mitochondrial genome, the hypoxia-induced switch from mitochondrial respiration to glycolysis or the metabolic reprogramming resulting from the loss-of-function of enzymes like fumarate and succinate dehydrogenases. How aerobic glycolysis and apoptosis resistance are linked remains to be elucidated. On the one hand, these alterations may be acquired independently by cancer cells during multistep oncogenesis. On the other hand, the suppression of the intrinsic apoptotic program may be achieved through mechanisms that directly lead to the Warburg phenotype. Cancer-specific mitochondrial alterations and bioenergetics may be taken advantage for the development of two novel classes of antineoplastic agents. A first approach would target glycolysis and/or revert the Warburg phenomenon, whereas a second approach would aim at inducing apoptosis by targeting mitochondrial proteins and membranes. In both instances, encouraging pre-clinical results have been obtained (4).

Given the role of mitochondria in oxygen consumption, metabolism and cell death regulation, alterations in mitochondrial function or dysregulation of cell death pathways contribute to the genesis and progression of cancer. Cancer cells exhibit an array of metabolic transformations induced by mutations leading to gain-of-function of oncogenes and loss-of-function of tumor suppressor genes that include increased glucose consumption, reduced mitochondrial respiration, increased reactive oxygen species generation and cell death resistance, all of which ensure cancer progression. Cholesterol metabolism is disturbed in cancer cells and supports uncontrolled cell growth. In particular, the accumulation of cholesterol in mitochondria emerges as a molecular component that orchestrates some of these metabolic alterations in cancer cells by impairing mitochondrial function. As a consequence, mitochondrial cholesterol loading in cancer cells may contribute, in part, to the Warburg effect stimulating aerobic glycolysis to meet the energetic demand of proliferating cells, while protecting cancer cells against mitochondrial apoptosis due to changes in mitochondrial membrane dynamics. Further understanding the complexity in the metabolic alterations of cancer cells, mediated largely through alterations in mitochondrial function, may pave the way to identify more efficient strategies for cancer treatment involving the use of small molecules targeting mitochondria, cholesterol homeostasis/trafficking and specific metabolic pathways (5).

It is a longstanding debate whether cancer is one disease or a set of very diverse diseases. The goal of this paper is to suggest strongly that most of (if not all) the hallmarks of cancer could be the consequence of the Warburg's effect. As a result of the metabolic impairment of the oxidative phosphorylation, there is a decrease in ATP concentration. To compensate the reduced energy yield, there is massive glucose uptake, anaerobic glycolysis, with an up-regulation of the Pentose Phosphate Pathway resulting in increased biosynthesis leading to increased cell division and local pressure. This increased pressure is responsible for the fractal shape of the tumor, the secretion of collagen by the fibroblasts and plays a critical role in metastatic spread. The massive extrusion of lactic acid contributes to the extracellular acidity and the activation of the immune system. The decreased oxidative phosphorylation leads to impairment in CO2 levels inside and outside the cell, with increased intracellular alkalosis and contribution of carbonic acid to extracellular acidosis-mediated by at least two cancer-associated carbonic anhydrase isoforms. The increased intracellular alkalosis is a strong mitogenic signal, which bypasses most inhibitory signals. Mitochondrial disappearance (such as seen in very aggressive tumors) is a consequence of mitochondrial swelling, itself a result of decreased ATP concentration. The transmembrane pumps, which extrude, from the mitochondria, ions, and water, are ATP-dependant. Therapy aiming at increasing both the number and the efficacy of mitochondria could be very useful (6).

A shift from respiration to fermentation is a common metabolic hallmark of cancer cells. As a result, glucose and glutamine become the prime fuels for driving the dysregulated growth of tumors. The simultaneous occurrence of "Press-Pulse" disturbances was considered the mechanism responsible for reduction of organic populations during prior evolutionary epochs. Press disturbances produce chronic stress, while pulse disturbances produce acute stress on populations. It was only when both disturbances coincide that population reduction occurred.

This general concept can be applied to the management of cancer by creating chronic metabolic stresses on tumor cell energy metabolism (press disturbance) that are coupled to a series of acute metabolic stressors that restrict glucose and glutamine availability while also stimulating cancer-specific oxidative stress (pulse disturbances). The elevation of non-fermentable ketone bodies protect normal cells from energy stress while further enhancing energy stress in tumor cells that lack the metabolic flexibility to use ketones as an

efficient energy source. Mitochondrial abnormalities and genetic mutations make tumor cells vulnerable metabolic stress.

The press-pulse therapeutic strategy for cancer management is illustrated with calorie restricted ketogenic diets (KD-R) used together with drugs and procedures that create both chronic and intermittent acute stress on tumor cell energy metabolism, while protecting and enhancing the energy metabolism of normal cells.

Optimization of dosing, timing, and scheduling of the press-pulse therapeutic strategy will facilitate the eradication of tumor cells with minimal patient toxicity. This therapeutic strategy can be used as a framework for the design of clinical trials for the non-toxic management of most cancers (7).

The potential role of the mitochondrial genome has recently attracted interest because of its high mutation frequency in tumors. Different aspects of mtDNA make it relevant for cancer's biology, such as it encodes a limited but essential number of genes for OXPHOS biogenesis, it is particularly susceptible to mutations, and its copy number can vary. Moreover, most ROS in mitochondria are produced by the electron transport chain. These characteristics place the mtDNA in the center of multiple signaling pathways, known as mitochondrial retrograde signaling, which modifies numerous key processes in cancer. Cybrid studies support that mtDNA mutations are relevant and exert their effect through a modification of OXPHOS function and ROS production. However, there is still much controversy regarding the clinical relevance of mtDNA mutations. New studies should focus more on OXPHOS dysfunction associated with a specific mutational signature rather than the presence of mutations in the mtDNA (8).

In the last years, metabolic reprogramming, fluctuations in bioenergetic fuels, and modulation of oxidative stress became new key hallmarks of tumor development. In cancer, elevated glucose uptake and high glycolytic rate, as a source of adenosine triphosphate, constitute a growth advantage for tumors. This represents the universally known Warburg effect, which gave rise to one major clinical application for detecting cancer cells using glucose analogs: the positron emission tomography scan imaging. Recent Advances: Glucose utilization and carbon sources in tumors are much more heterogeneous than initially thought. Indeed, new studies emerged and revealed a dual capacity of tumor cells for glycolytic and oxidative phosphorylation (OXPHOS) metabolism. OXPHOS metabolism, which relies predominantly on mitochondrial respiration, exhibits fine-tuned regulation of respiratory chain complexes and enhanced antioxidant response or detoxification capacity.

OXPHOS-dependent cancer cells use alternative oxidizable substrates, such as glutamine and fatty acids. The diversity of carbon substrates fueling neoplastic cells is indicative of metabolic heterogeneity, even within tumors sharing the same clinical diagnosis. Metabolic switch supports cancer cell stemness and their bioenergy-consuming functions, such as proliferation, survival, migration, and invasion. Moreover, reactive oxygen species-induced mitochondrial metabolism and nutrient availability are important for interaction with tumor microenvironment components. Carcinoma-associated fibroblasts and immune cells participate in the metabolic interplay with neoplastic cells. They collectively adapt in a dynamic manner to the metabolic needs of cancer cells, thus participating in tumorigenesis and resistance to treatments.

Characterizing the reciprocal metabolic interplay between stromal, immune, and neoplastic cells will provide a better understanding of treatment resistance (9).

In differentiated normal cells, the conventional route of glucose metabolism involves glycolysis, followed by the citric acid cycle and electron transport chain to generate usable energy in the form of adenosine triphosphate (ATP). This occurs in the presence of oxygen. In hypoxic conditions, normal cells undergo anaerobic glycolysis to yield significantly less energy producing lactate as a product. As first highlighted in the 1920s by Otto Warburg, the metabolism exhibited by tumor cells involves an increased rate of aerobic glycolysis, known as the Warburg effect. In aerobic glycolysis, pyruvate molecules yielded from glycolysis are converted into fewer molecules of ATP even in the presence of oxygen. Evidence indicates that the reasons as to why tumor cells undergo aerobic glycolysis include: (1) the shift in priority to accumulate biomass rather than energy production, (2) the evasion of apoptosis as fewer reactive oxygen species are released by the mitochondria and (3) the production of lactate to further fuel growth of tumors (10).

As stated by Otto Warburg nearly a century ago, cancer is a metabolic disease, a fermentation caused by malfunctioning mitochondria, resulting in increased anabolism and decreased catabolism. Treatment should, therefore, aim at restoring the energy yield. To decrease anabolism, glucose uptake should be reduced (ketogenic diet). To increase catabolism, the oxidative phosphorylation should be restored. Treatment with a combination of α-lipoic acid and hydroxycitrate has been shown to be effective in multiple animal models. This treatment, in combination with conventional chemotherapy, has yielded extremely encouraging results in glioblastoma, brain metastasis and lung cancer. Randomized trials are necessary to confirm these preliminary data. The major limitation is the fact that the combination of α-lipoic acid and hydroxycitrate can only be effective if the mitochondria are still present and/or functional. That may not be the case in the most aggressive tumors. The increased intracellular alkalosis is a strong mitogenic signal, which bypasses most inhibitory

signals. Concomitant correction of this alkalosis may be a very effective treatment in case of mitochondrial failure (11).

INTRALIPID

Accidental intravascular or high-dose injection of local anesthetics (LA) can result in serious, potentially life-threatening complications. Indeed, adequate supportive measures and the administration of lipid emulsions are required in such complications. The study's objectives were threefold: (i) evaluate the myocardial toxicity of levobupivacaine when administered intravenously; (ii) investigate levobupivacaine toxicity on cardiomyocytes mitochondrial functions and cellular structure; (iii) assess the protective effects of a lipid emulsion in the presence or absence of myocardial ischemia. Domestic pigs randomized into two groups of 24 animals each, with either preserved coronary circulation or experimental myocardial ischemia. Six animals from each group received either: (i) single IV injection of saline, (ii) lipid emulsion (Intralipid(®)), (iii) levobupivacaine, (iv) combination levobupivacaine-Intralipid(®) . Serially measured endpoints included: heart rate, duration of the monophasic action potentials (dMAP), mean arterial pressure, and peak of the time derivative of left ventricular pressure (LV dP/dtmax). In addition, the following cardiomyocytes mitochondrial functions were measured: reactive oxygen species (ROS) production, oxidative phosphorylation, and calcium retention capacity (CRC) as well as the consequences of ROS production on lipids, proteins, and DNA. IV injection of levobupivacaine induced sinus bradycardia and reduced dMAP and LV dP/dtmax . At the mitochondrial level, oxygen consumption and CRC were decreased. In contrast, ROS production was increased leading to enhanced lipid peroxidation and structural alterations of proteins and DNA. Myocardial ischemia was associated with global worsening of all changes. Intralipid(®) quickly improved haemodynamics. However, beneficial effects of Intralipid(®) were less clear after myocardial ischemia (12).

We hypothesized that acute lipid-induced insulin resistance would be attenuated in high-oxidative muscle of lean trained (LT) endurance athletes due to their enhanced metabolic flexibility and mitochondrial capacity. Lean sedentary (LS), obese sedentary (OS), and LT participants completed two hyperinsulinemic euglycemic clamp studies with and without (glycerol control) the coinfusion of Intralipid. Metabolic flexibility was measured by indirect calorimetry as the oxidation of fatty acids and glucose during fasted and insulin-stimulated conditions, the latter with and without lipid oversupply. Muscle biopsies were obtained for mitochondrial and insulin-signaling studies. During hyperinsulinemia without lipid, glucose infusion rate (GIR) was lowest in OS due to lower rates of nonoxidative glucose disposal (NOGD), whereas state 4 respiration was increased in all groups. Lipid infusion reduced GIR similarly in all subjects and reduced state 4 respiration. However, in LT subjects, fat oxidation was higher with lipid oversupply, and although glucose oxidation was reduced, NOGD was better preserved compared with LS and OS subjects. Mitochondrial performance was positively associated with better NOGD and insulin sensitivity in both conditions. We conclude that enhanced mitochondrial performance with exercise is related to better metabolic flexibility and insulin sensitivity in response to lipid overload (13).

Intralipid® administration at reperfusion elicits protection against myocardial ischemia-reperfusion injury. However, the underlying mechanisms are not fully understood.

Sprague-Dawley rat hearts were exposed to 15 min of ischemia and 30 min of reperfusion in the absence or presence of Intralipid® 1% administered at the onset of reperfusion. In separate experiments, the reactive oxygen species (ROS) scavenger N-(2-mercaptopropionyl)-glycine was added either alone or with Intralipid®. Left ventricular work and activation of Akt, STAT3, and ERK1/2 were used to evaluate cardioprotection. ROS production was assessed by measuring the loss of aconitase activity and the release of hydrogen peroxide using Amplex Red. Electron transport chain complex activities and proton leak were measured by high-resolution respirometry in permeabilized cardiac fibers. Titration experiments using the fatty acid intermediates of Intralipid® palmitoyl-, oleoyl- and linoleoylcarnitine served to determine concentration-dependent inhibition of complex IV activity and mitochondrial ROS release.

Intralipid® enhanced postischemic recovery and activated Akt and Erk1/2, effects that were abolished by the ROS scavenger N-(2-mercaptopropionyl)glycine. Palmitoylcarnitine and linoleoylcarnitine, but not oleoylcarnitine concentration-dependently inhibited complex IV. Only palmitoylcarnitine reached high tissue concentrations during early reperfusion and generated significant ROS by complex IV inhibition. Palmitoylcarnitine (1 μM), administered at reperfusion, also fully mimicked Intralipid®-mediated protection in an N-(2-mercaptopropionyl)-glycine -dependent manner.

Our data describe a new mechanism of postconditioning cardioprotection by the clinically available fat emulsion, Intralipid®. Protection is elicited by the fatty acid intermediate palmitoylcarnitine, and involves inhibition of complex IV, an increase in ROS production and activation of the RISK pathway (14).

We have recently shown that postischemic administration of intralipid protects the heart against ischemia-reperfusion injury. Here we compared the cardioprotective effects of intralipid with cyclosporine-A, a potent inhibitor of the mitochondrial permeability transition pore opening.

In vivo rat hearts or isolated Langendorff-perfused mouse hearts were subjected to ischemia followed by reperfusion with intralipid (0.5%, 1% and 2% ex-vivo, and 20% in vivo), cyclosporine-A (0.2 μM, 0.8 μM, and 1.5 μM ex- vivo and 10 mg/kg in vivo), or vehicle. The hemodynamic function, infarct size, calcium retention capacity, mitochondrial superoxide production, and phosphorylation levels of protein kinase B (Akt)/glycogen synthase kinase-3β (GSK-3β) were measured. The values are mean ± SEM.

Administration of intralipid at reperfusion significantly reduced myocardial infarct size compared with cyclosporine-A in vivo (infarct size/area at risk)%: 22.9 ± 2.5% vs. 35.2 ± 3.5%; P = 0.030, n = 7/group). Postischemic administration of intralipid at its optimal dose (1%) was more effective than cyclosporine-A (0.8 μM) in protecting the ex vivo heart against ischemia-reperfusion injury, as the rate pressure product at the end of reperfusion was significantly higher (mmHg · beats/min: 12,740 ± 675 [n = 7] vs. 9,203 ± 10,781 [n = 5], P = 0.024), and the infarct size was markedly smaller (17.3 ± 2.9 [n = 7] vs. 29.2 ± 2.7 [n = 5], P = 0.014). Intralipid was as efficient as cyclosporine-A in inhibiting the mitochondrial permeability transition pore opening (calcium retention capacity = 280 ± 8.2 vs. 260.3 ± 2.9 nmol/mg mitochondria protein in cyclosporine-A, P = 0.454, n = 6) and in reducing cardiac mitochondrial superoxide production. Unlike intralipid, which increased phosphorylation of Akt (6-fold) and GSK-3β (5-fold), cyclosporine-A had no effect on the activation of these prosurvival kinases.

Although intralipid inhibits the opening of the mitochondrial permeability transition pore as efficiently as cyclosporine-A, intralipid is more effective in reducing the infarct size and improving the cardiac functional recovery (15).

Free fatty acid (FFA)- and obesity-induced insulin resistance has been associated with disturbed mitochondrial function. Elevated plasma FFA can impair insulin-induced increase of adenosine triphosphate synthesis and downregulate the expression of genes important in the biogenesis of mitochondria in human skeletal muscle. Whether FAs have a direct effect on intrinsic mitochondrial capacity remains to be established. Therefore, we measured ex vivo mitochondrial respiratory capacity in human skeletal muscle after exposure to hyperinsulinemia and high levels of plasma FFA. Nine healthy lean men were studied during a 6-hour hyperinsulinemic (600 pmol/L) euglycemic clamp with concomitant infusion of Intralipid (Fresensius Kabi Nederland, Den Bosch, the Netherlands) (FFA clamped at 0.5 mmol/L) or saline. Mitochondrial respiratory capacity was measured by high-resolution respirometry in permeabilized muscle fibers using an Oxygraph (OROBOROS Instruments, Innsbruck, Austria). Each participant served as his own control. Peripheral glucose uptake (rate of disappearance) was significantly lower during infusion of the lipid emulsion compared with the control saline infusion (68 μmol/kg·min [saline] vs 40 μmol/kg·min [lipid], P = .008). However, adenosine diphosphate-stimulated and maximal carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone-stimulated uncoupled respiration rates were not different in permeabilized skeletal muscle fibers after exposure to high levels of FFA compared with the control condition. We conclude that short-term elevation of FFA within the physiological range induces insulin resistance but does not affect intrinsic mitochondrial capacity in skeletal muscle in humans (16).

Local anesthetics are used broadly to prevent or reverse acute pain and treat symptoms of chronic pain. Local anesthetic-induced cardiotoxic reaction has been considered the accidental event without currently effective therapeutic drugs except for recently reported intralipid infusion whose possible mechanism of action is not well known.

Cardiolipin, an anionic phospholipid, plays a key role in determining mitochondrial respiratory reaction, fatty acid metabolism and cellular apoptosis. Mitochondrial energy metabolism dysfunction is suggested as associated with local anesthetic cardiotoxicity, from an in vitro study report that the local anesthetic cardiotoxicity may be due to the strong electrostatic interaction of local anesthetics and cardiolipin in the mitochondria membrane, although there is a lack for experimental evidence. Herein we hypothesized that local anesthetic-cardiolipin interactions were the major determinant of local anesthetic-associated cardiotoxic reaction, established by means of theoretic and structural biological methods.

The interaction between local anesthetic and mitochondrial cardiolipin may be the underlying mechanism for cardiotoxicity affecting its energy metabolism and electrostatic status (17).

We have previously shown that lack of thioredoxin-interacting protein (TXNIP) protects against diabetes and glucotoxicity-induced beta-cell apoptosis. Because the role of TXNIP in lipotoxicity is unknown, the goal of the present study was to determine whether TXNIP expression is regulated by fatty acids and whether TXNIP deficiency also protects beta-cells against lipoapoptosis. To determine the effects of fatty acids on beta-cell TXNIP expression, INS-1 cells and isolated islets were incubated with/without palmitate and rats underwent cyclic infusions of glucose and/or Intralipid prior to islet isolation and analysis by quantitative real-time RT-PCR and immunoblotting. Using primary wild-type and TXNIP-deficient islets, we then assessed the effects of palmitate on apoptosis (transferase-mediated dUTP nick-end labeling [TUNEL]), mitochondrial death pathway (cytochrome c release), and endoplasmic reticulum (ER) stress (binding protein [BiP], C/EBP

homologous protein [CHOP]). Effects of TXNIP deficiency were also tested in the context of staurosporine (mitochondrial damage) or thapsigargin (ER stress).

Glucose elicited a dramatic increase in islet TXNIP expression both in vitro and in vivo, whereas fatty acids had no such effect and, when combined with glucose, even abolished the glucose effect. We also found that TXNIP deficiency does not effectively protect against palmitate or thapsigargin-induced beta-cell apoptosis, but specifically prevents staurosporine- or glucose-induced toxicity.

Our results demonstrate that unlike glucose, fatty acids do not induce beta-cell expression of proapoptotic TXNIP. They further reveal that TXNIP deficiency specifically inhibits the mitochondrial death pathway underlying beta-cell glucotoxicity, whereas it has very few protective effects against ER stress-mediated lipoapoptosis (18).

To investigate the mechanism of beta-cell dysfunction induced by glucolipotoxicity in high fat-fed obese rats : Eighteen high-fat obese male Wistar rats were assigned into 3 groups and underwent 48-hour infusion through the jugular vein with normal saline (n=6), 20% intralipid + heparin (FFA group, n=6), or 25%glucose +20% intralipid + heparin (GS-FFA group, n=6). The plasma beta-hydroxybutyric acid (beta-HBA) was measured before and at the end of the infusion. After the infusion, the rats were sacrificed following an intravenous glucose tolerance test (IVGTT) to remove the tail of the pancreas for detection of apoptotic islet cells using TUNEL method. Immunohistochemical staining was performed to detect the expression of cytochrome c (cyt c), apoptosis-inducing factor (AIF), caspase-9 and caspase-3 in the islet cells**.**

At the end of the infusion, all the rats exhibited increased plasma beta-HBA levels, which was the highest in the GS-FFA group ($P<0.05$). IVGTT performed after the infusion showed a significantly lower insulinogenic index in GS-FFA group than that in NS and FFA groups. Greater number of apoptotic islet cells was found in the GS-FFA group than in the FFA and NS groups ($P<0.05$), and the islets had significantly higher levels of cyt c, AIF, caspase-9 and caspase-3 in the former group than in the latter two groups ($P<0.05$).

Hyperglycemia and high free fatty acid level synergistically impair insulin secretions to cause ketone overproduction in high fat-fed obese rats. The beta-cell dysfunction due to glucolipotoxicity is associated with increased beta-cell apoptosis and activation of mitochondrial apoptotic pathway (19).

To investigate pyruvate dehydrogenase (PDH)-E1alpha subunit phosphorylation and whether free fatty acids (FFAs) regulate PDH activity, seven subjects completed two trials: saline (control) and intralipid/heparin (intralipid). Each infusion trial consisted of a 4-h rest followed by a 3-h two-legged knee extensor exercise at moderate intensity. During the 4-h resting period, activity of PDH in the active form (PDHa) did not change in either trial, yet phosphorylation of PDH-E1alpha site 1 (PDH-P1) and site 2 (PDH-P2) was elevated in the intralipid compared with the control trial. PDHa activity increased during exercise similarly in the two trials. After 3 h of exercise, PDHa activity remained elevated in the intralipid trial but returned to resting levels in the control trial. Accordingly, in both trials PDH-P1 and PDH-P2 decreased during exercise, and the decrease was more marked during intralipid infusion. Phosphorylation had returned to resting levels at 3 h of exercise only in the control trial. Thus, an inverse association between PDH-E1alpha phosphorylation and PDHa activity exists. Short-term elevation in plasma FFA at rest increases PDH-E1alpha phosphorylation, but exercise overrules this effect of FFA on PDH-E1alpha phosphorylation leading to even greater dephosphorylation during exercise with intralipid infusion than with saline (20).

We have developed a stable isotope breath test to trace physiological remnant metabolism. Validity of the test depends on the injected lipid emulsion mimicking chylomicron remnant (CR) clearance and on subsequent metabolism of the emulsion cholesteryl ester (CE). Oxidation of CE fatty acids could involve both mitochondrial and peroxisomal pathways. In the present studies various agents were used to inhibit the binding of remnants, CE hydrolysis or mitochondrial fatty acid oxidation. Treatment of mice with suramin or lactoferrin markedly delayed the clearance and metabolism of remnants as shown by the significantly lower enrichment of 13CO(2) in the breath when compared with untreated mice. In hepatectomized rats injected with remnant-like emulsions, enrichment with 13CO(2) was virtually abolished. Treatment of mice with chloroquine or rats with methyl palmoxirate (an inhibitor of mitochondrial fatty acid oxidation) markedly impaired the recovery of label in the breath. Compared with mice fasted overnight, Intralipid by gavage decreased the breath enrichment with 13CO(2) consistent with competition between endogenous CR and the injected emulsion particles. These findings show that the breath test reliably measures the metabolism of CR and that CE fatty acid is metabolised by mitochondrial pathways (21).

Diminishing oxidative stress may protect the heart against ischaemia-reperfusion injury by preventing opening of the mitochondrial permeability transition (MPT) pore. The general anaesthetic agent propofol, a free radical scavenger, has been investigated for its effect on the MPT and its cardioprotective action following global and cardioplegic ischaemic arrest.

Isolated perfused Wistar rat hearts were subjected to either warm global ischaemia (Langendorff) or cold St. Thomas' cardioplegia (working heart mode) in the presence or absence of propofol. MPT pore opening

was determined using [3H]-2-deoxyglucose-6-phosphate ([3H]-DOG-6P) entrapment. The respiratory function of isolated mitochondria was also determined for evidence of oxidative stress.

Propofol (2 micrograms/ml) significantly improved the functional recovery of Langendorff hearts on reperfusion (left ventricular developed pressure from 28.4 +/- 6.2 to 53.3 +/- 7.3 mmHg and left ventricular end diastolic pressure from 52.9 +/- 4.3 to 37.5 +/- 3.9 mmHg). Recovery was also improved in propofol (4 micrograms/ml) treated working hearts following cold cardioplegic arrest. External cardiac work on reperfusion improved from 0.42 +/- 0.05 to 0.60 +/- 0.03 J/s, representing 45-64% of baseline values, when compared to controls ($P < 0.05$). Propofol inhibited MPT pore opening during reperfusion, [3H]-DOG-6P entrapment being 16.7 vs. 22.5 ratio units in controls ($P < 0.05$). Mitochondria isolated from non-ischaemic, propofol-treated hearts exhibited increased respiratory chain activity and were less sensitive to calcium-induced MPT pore opening.

Propofol confers significant protection against global normothermic ischaemia and during cold cardioplegic arrest. This effect is associated with less opening of mitochondrial MPT pores, probably as a result of diminished oxidative stress. Propofol may be a useful adjunct to cardioplegic solutions in heart surgery (22).

We studied the variations arising in plasma and liver lipids after intravenous (i.v.), intraperitoneal (IP), and intragastric (IG) administration of a fat overdose on the order of 4 g.kg-1 body wt.day-1 in the form of Intralipid (ITL) 20% to 33 New Zealand rabbits for 15 days. The control group was submitted for surgery but did not receive an ITL supplement. The results show weight gain in all animals and normal liver enzyme values. There was an increase in plasma lipids in groups supplemented by the parenteral route (i.v. and IP), and fatty acids showed a similar distribution, in terms of percentages, to that for ITL. In liver tissue, there was an increase in the fractions related to ethanolamine and a decrease in phospholipids of choline and serine. In the i.v. group, neutral lipids predominated compared with other groups. The livers of all supplemented animals (i.v., IP, and IG) showed a higher content of stearic and linoleic acid and a reduction in oleic acid. Study with optical microscopy showed a microvacuolization affecting the three areas of the hepatic acini in the i.v. group, seen with electron microscopy as vacuoles lacking membranes and surrounded by mitochondria. In conclusion, there is an increase in hepatic steatosis in parenteral groups and a greater deposit of neutral lipids in the i.v. group, related to the administration route, without biochemical signs of liver dysfunction (23).

The metabolism of Intralipid (intravenously injected) was studied in rats fasted for 48 h. At all doses used, the Intralipid triacylglycerols disappeared rapidly from circulation and concomitantly the hepatic content of triacylglycerols and the level of circulating ketone bodies increased, indicating an active metabolism of Intralipid by the liver. To study this possibility further we used an ultrastructural approach. In rats given Intralipid we detected numerous lipid particles in the spaces of Disse, retained in the interdigitations of the hepatocyte. There were also lipid particles attached to the luminal surface of the endothelial cells. Small lipid particles were seen in close contact with endocytic vesicles internalized into hepatocytes but were present mainly in endothelial cells. Inside the endothelial cells, the endocytic vesicles were detected in contact with lysosomes. Inside hepatocytes, a process of sterification seemed to occur in the endoplasmic reticulum as deduced from the presence of small lipid droplets with ill-defined outlines. Large lipid droplets were seen in close contact with mitochondria, indicating a mitochondrial uptake and metabolism of fatty acids to synthesize and release ketone bodies. The possible role of lipoprotein lipase in the liver for the hepatic uptake of Intralipid particles is discussed (24).

Effects of high plasma free fatty acids (FFA) on the free radical formation of myocardial mitochondria, isolated from normal and ischemic dog hearts, were studied by electron spin resonance (ESR) spectrometry. Free radical concentrations in state 4 respiration were used for the evaluation of the function in the mitochondria in this study. High plasma FFA levels were induced either by intravenous injection of Intralipid and heparin, or by infusion of norepinephrine. Ischemic hearts were induced by inserting a Cournand's 7F catheter into the left coronary artery under fluoroscopic control. Exogenous high plasma FFA induced by Intralipid and heparin caused the decrease of free radicals in state 4 respiration in the mitochondria isolated from normal and ischemic dog hearts. Endogenous high FFA induced by continuous infusion of norepinephrine also caused the decrease of free radicals. On the other hand, nicotinic acid prevented the decrease of free radicals as well as the rise of plasma FFA by the norepinephrine infusion. These results suggest that high plasma FFA itself, whether it may be exogenous or endogenous, may impair the oxidative phosphorylation of the mitochondria isolated from normal and ischemic hearts (25).

Low-density lipoprotein (LDL) could be used as a carrier of chemotherapeutic agents to neoplastic cells that overexpress LDL receptors (rLDL), but LDL is difficult to obtain and handle. Recently, it was observed that a protein-free emulsion resembling the lipid portion of LDL (LDE) behave like native LDL when injected into the bloodstream. In this study, the evidence that LDE is taken up by rLDL was expanded by comparing LDL and LDE plasma decay curves in rabbits and by competition experiments with lymphocytes. To verify whether LDE could be removed from the plasma by neoplastic cells with increased rLDL, LDE labeled

with 14Ccholesteryl ester was injected into 14 patients with acute myeloid leukemia (AML) and into 7 with acute lymphocytic leukemia (ALL). In AML rLDL expression is increased but in ALL it is normal. LDE plasma fractional clearance rate (FCR, in h-1) was calculated from the remaining radioactivity measured in plasma samples collected during 24 h following injection. LDE FCR was 3-fold greater in AML than in ALL patients 0.192 +/- 0.210 (SD) and 0.066 +/- 0.033 h-1, respectively, $P < 0.035$. When LDE injection was repeated in 9 AML patients in hematological remission, LDE FCR diminished 66% compared to the pretreatment values (from 0.192 +/- 0.210 to 0.065 +/- 0.038 h-1, $P < 0.02$), so that it could be estimated that nearly 66% of the emulsion was taken up by AML cells and only 34% by the normal tissues. As expected, LDE FCR was unchanged in 4 patients with ALL in hematological remission (0.069 +/- 0.044 h-1). Gamma camera images obtained 6 h after the injection of 99mTc-label LDE into one patient with ALL showed biodistribution similar to that of LDL. In one AML patient LDE was comparatively more concentrated over the areas corresponding to the bone marrow infiltrated by AML cells. Our results indicate that LDE FCR is increased in a disease known to contain malignant cells that overexpress rLDL, suggesting that LDE is taken up by malignant cells with increased rLDL (26).

Previously, it was shown that a lipidic emulsion (LDE) composed of phospholipids and cholesterol esters which binds to low-density lipoprotein (LDL) receptors may concentrate in acute myeloid leukemia cells. In this study, we aimed to verify whether LDE also has the ability to concentrate in malignant ovarian cancer after being injected into the blood circulation of the patients.

Three groups of women scheduled for surgery were included in the survey: 13 bearing malignant tumors, 9 with benign ovarian tumors, and 13 without ovarian tumor who were scheduled to undergo oophorectomy due to malignant disease of the uterine cervix or endometrium. On the day prior to surgery they were injected with LDE labeled with [(14)C]cholesteryl oleate. Specimens of tumors and normal ovaries excised during surgery were lipid extracted and analyzed for radioactivity counting. Results were expressed in radioactive count (cpm) per gram of tissue.

The mean of the uptakes of the emulsion radioactivity by the malignant tumors was roughly eightfold greater when compared with that of the contralateral normal ovaries (2261 +/- 1444 and 275 +/- 137 cpm/g, respectively, $P < 0.012$), benign tumors, and normal ovaries of the patients without ovarian tumors.

LDE has the ability to concentrate in malignant ovarian tumor tissue. Therefore, it can be used as a vehicle to direct cytotoxic drugs against malignant ovarian tumors, thus diminishing the side effects of chemotherapy (27).

Overexpression of low-density lipoprotein (LDL) receptors occurs in several cancer cell lines and offers a unique strategy for drug targeting by using LDL as vehicle. However, the native lipoprotein is difficult to obtain and handle. Previously, we showed that a lipidic emulsion (LDE) similar to the lipid structure of native LDL may bind to LDL receptors and be taken up by acute myelocytic leukemia cells. We also showed that LDE can also concentrate in ovarian cancer tissue. In this study, we tested whether LDE is taken up by breast carcinoma.

LDE labeled with (99m)Tc was injected into 18 breast cancer patients, and nuclear medicine images of the tumor and metastatic sites were acquired. Subsequently, LDE labeled with [3H]cholesteryl oleate was intravenously injected into 14 breast cancer patients 24-30 h before total mastectomy procedure. Fragments of normal and of breast cancer tissue excised during surgery were lipid extracted with chloroform/methanol and their radioactivity was measured in a scintillation solution.

(99m)Tc-LDE images of the primary tumor and of metastasis sites were obtained in all 18 breast cancer patients. As directly measured in the tumor and in the normal mammary tissue, the amount of the emulsion radioactive label in the tumor was 4.5 times greater than in the normal tissue (range 1.2- to 8.8-fold).

LDE concentrates much more in malignant breast tumor tissue than in the normal tissue. Thus it has potential to carry drugs or radionuclides directed against mammary carcinoma cells for diagnostic or therapeutic purposes (28).

Conclusion

It is suggested that based on the Warburg effect and the combination of Intralipid is a new modality in cancer treatment.

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