# Discovering the Controls of Fatty Acid Oxidation and Ketogenesis

## University of Texas Southwestern Medical Center

Internal Medicine Grand Rounds

November 8, 2013

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# Dr. Daniel W. Foster

**John Denis McGarry, Ph.D. Distinguished Chair in Diabetes and Metabolic Research**
**Division of Endocrinology and Metabolism**


I decided to give this Grand Rounds in honor of Dr. John Denis McGarry, my first fellow and longtime colleague. He died from a glioblastoma multiforme in 2002. He was an outstanding scientist and a wonderful teacher.

Fatty acid oxidation and ketogenesis are crucial in normal life and in disease. In this discussion I will cover the early hypotheses of ketogenesis; hormonal control of ketogenesis; the role of carnitine in fatty acid oxidation and ketogenesis; the carnitine palmitoyltransferase system; malonyl CoA regulation of fatty acid oxidation and ketogenesis; the enzymes; and clinical implications in normal life and disease.

Purpose/Overview:
The initial studies of fatty acid diagnosis and ketogenesis were addressed by Nobel Laureates and other scientists. It was actually more complicated. I will review the major advances. Fatty acid oxidation and ketogenesis is life-saving in everyday life. Paradoxically, death can occur if fatty acid oxidation is absent or, on the other hand, if its overactive. I will describe briefly a patient with systemic carnitine deficiency and another with diabetic ketoacidosis.

Objectives:
1. To understand the history of the initial attempt to understand the mechanism of ketosis.
2. To understand the role of carnitine in fatty acid oxidation.
3. To understand the role of malonyl Co-A in regulating fatty acid oxidation and ketogenesis.
4. To understand why ketones are crucial in the brain.
5. To understand why carnitine deficiency is potentially fatal in children.

# Discovering the Controls of Fatty Acid Oxidation and Ketogenesis

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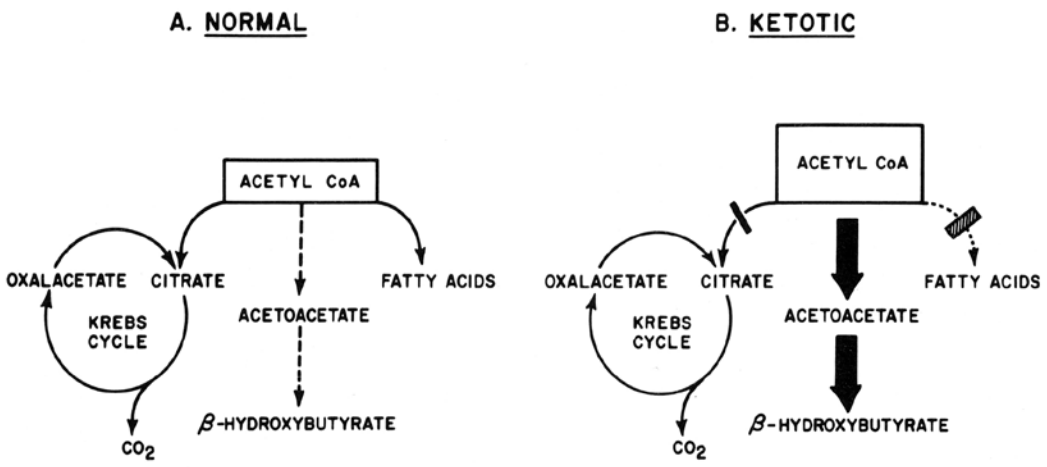
1. Early hypotheses of ketogenesis

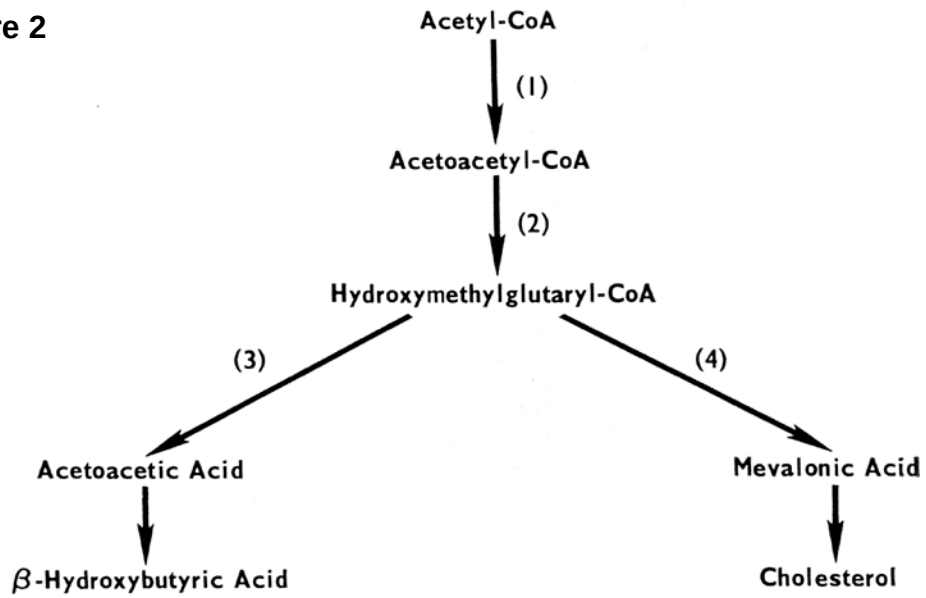
   It was widely believed early by serious scientists that ketogenesis was the consequence of the inability of acetyl CoA to enter the tricarboxylic acid cycle because of a deficiency of oxaloacetate with a similar block into fatty acid synthesis (1,2)

**Figure 1**



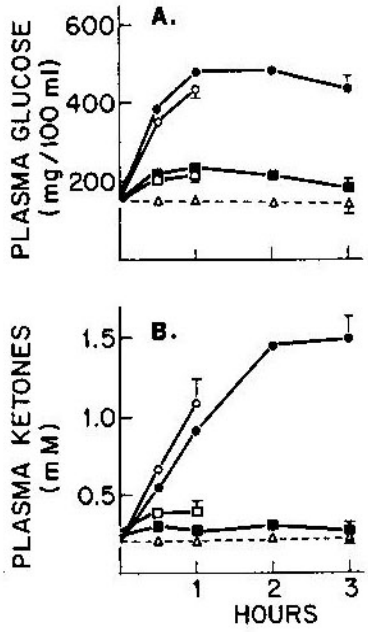

It was thought that the elevated acetyl CoA concentration was then converted to acetoacetic acid and β-hydroxybutyric acid. However, it was then shown that hepatic acetyl CoA was not elevated (3). It turned out that ketone synthesis was through 3-hydroxymethyl glutaryl-CoA, the precursor of both ketones and cholesterol(4).

**Figure 2**



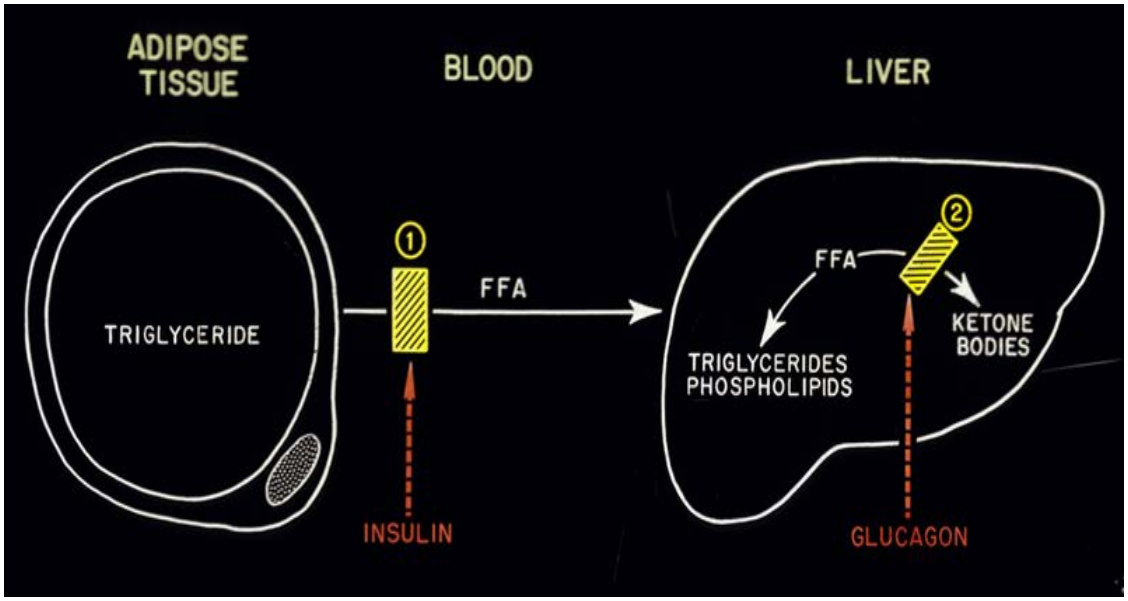

2. <u>Hormonal control</u>
   For years the assumption was that insulin was the primary hormone controlling metabolism. If deficient, as occurs in fasting, ketosis would occur, but not ketoacidosis. In 1975 studies by Drs. Roger Unger and Lelio Orci (5) showed that diabetes mellitus was a bi-hormonal disease, a relative or absolute deficiency of insulin and a relative or absolute elevation of glucagon. If one infuses anti-insulin antibodies and glucagon into fed rats, the plasma glucose increases and plasma ketones rise abruptly.

**Figure 3**



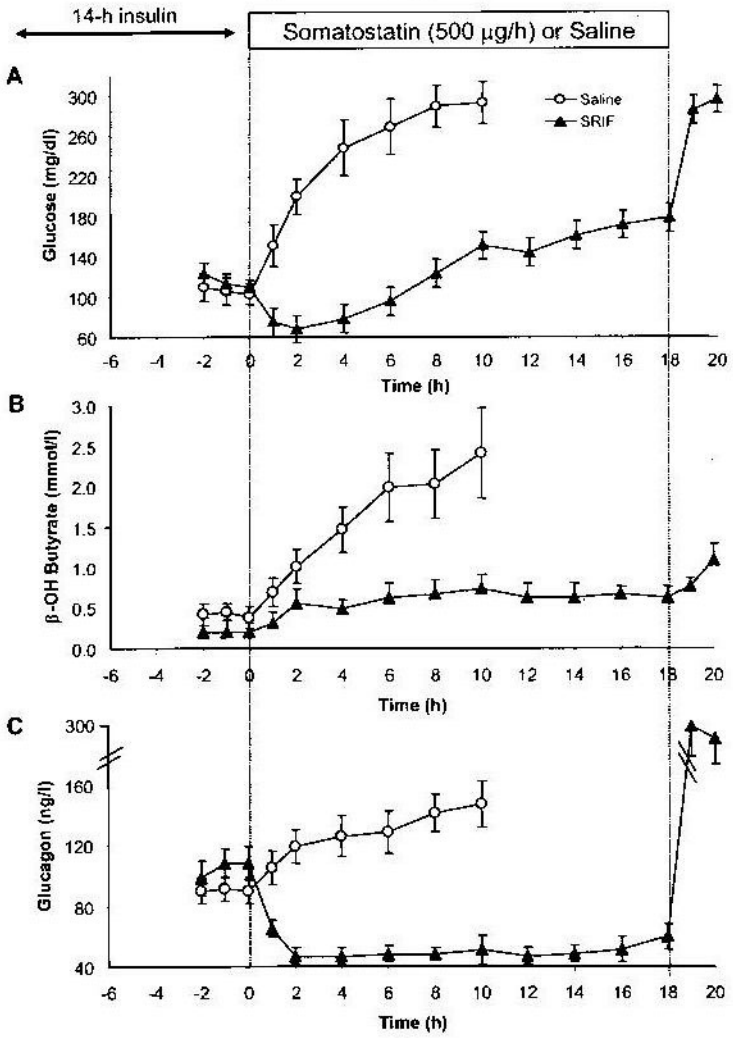
When we infused glucagon into non-ketotic rats and then infused triglyceride, they immediately became ketotic(6). This led to the conclusion that glucagon activated hepatic fatty acid oxidation while insulin deficiency delivered the substrate, long chain fatty acids, for ketone formation.

**Figure 4**




Confirmation in humans was shown by Gerich, *et al* (7), who infused somatostatin into patients with diabetic ketoacidosis to lower glucagon and reversed hyperglycemia and ketoacidosis. When somatostatin was stopped, hyperglycemia and ketoacidosis returned.

**Figure 5**




3. <u>The role of carnitine in fatty acid oxidation and ketogenesis</u>
   The first study suggesting carnitine as important in fatty acid oxidation and ketogenesis was published by Fitz and Yoe in 1963 (8). We developed a new method for measuring carnitine (9). We showed that carnitine increased rapidly with glucagon infusion, fasting and alloxan diabetic rats (10).

**Figure 6**

| Treatment | Ketone production from oleate, μmol/100 g body wt per 30 min | Free carnitine | Total carnitine |
|---|---|---|---|
| | | nmol/g wet wt of liver | |
| Fed (8) | 26 ± 3 | 40 ± 5 | 102 ± 10 |
| Fed, glucagon 3 hr (6) | 87 ± 5 | 68 ± 8 | 220 ± 13 |
| Fasted (6) | 118 ± 8 | 70 ± 5 | 228 ± 13 |
| Alloxan diabetic (6) | 192 ± 10 | 172 ± 12 | 416 ± 6 |

Carnitine levels were higher in dogs and humans than in rats.

4. <u>The carnitine palmitoyltransferase system</u>
   The carnitine palmitoyltransferase system is now well known and shown in ***Figure 7***.

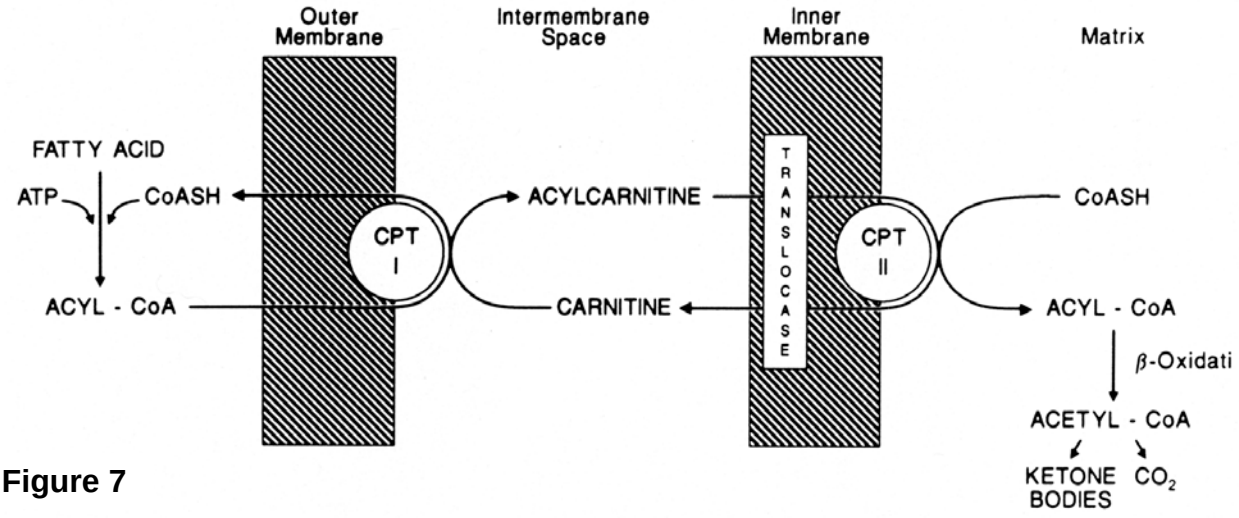



**Figure 7**

CPT1 is in the outer membrane of the mitochondria and CPTII is in the inner membrane. Fatty acyl CoA is transferred into triglyceride in the fed state and enters fatty acid β-oxidation in the fasted state or in poorly controlled type 1 diabetes. We originally thought that the initiating event was blockade of triglyceride synthesis. That would allow long-chain fatty acyl CoA to enter the mitochondrion. CPT 1 splits off CoA and attaches carnitine. Acyl carnitine is translocated to CPTII which exchanges carnitine for CoASH, allowing β-oxidation. In perfused isolated rat livers we tested an inhibitor of fatty acid β-oxidation, (+) deconylacylcarnitine. The substrate was 1-$^{14}$C oleic acid. In the fasted state, decreased liver lipids were converted to ketones. When (+dc) was added, liver triglycerides were immediately built up with ketones being reversed. This proved that the control was on β-oxidation, not triglyceride synthesis (11)

**Figure 8** **1 – $^{14}$C oleic acid metabolism (30 minutes) % recovered**

| | Ketones | Liver Lipids | Total |
|---|---|---|---|
| **Control** | **1.4** | **31.4** | **97.5** |
| **Fasted*** | **15.6** | **14.2** | **89.5** |
| **Fasted (+DC)*** | **0.9** | **27.9** | **92.5** |

5. Malonyl-CoA regulation of fatty acid oxidation and ketogenesis

One of the most important discoveries was the identification of malonyl-CoA as the regulator of fatty acid oxidation and ketogenesis (12).

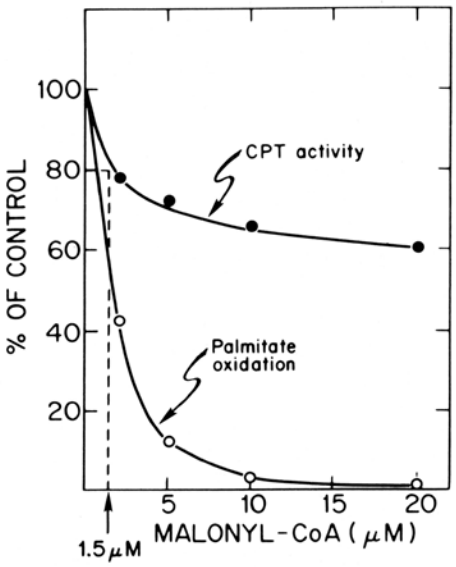



**Figure 9**

We next showed that CPT1 was the site of the inhibition by malonyl-CoA (13). We developed a radioisotope method of measuring malonyl-CoA that was more accurate than other assays (14, 15). Concentrations were higher in the fed state and fell in fasting. This was true in the liver, heart and muscle.

**Figure 10**

**Table II.** Malonyl-CoA Content of Various Rat Tissues

| State of animal | Malonyl-CoA content (nmol/g wet wt) | | |
|---|---|---|---|
| | Liver | Heart | Skel. muscl |
| Fed | 7.5 | 4.6 | 2.1 |
| Fasted 24 h | 1.7 | 1.4 | 0.7 |
| Fasted 48 h | 1.7 | 1.3 | 0.4 |

Malonyl-CoA does not inhibit medium chain fatty acids, only long-chain fatty acids.

6. <u>The Enzymes</u>

We cloned and sequenced CPT1 and CPTII (16-18). I won't cover this in detail. CPT1 and CPTII are distinct proteins. CPT1 exists in two isoforms, liver and muscle. Liver CPT1 requires much higher malonyl-CoA levels than muscle CPT1. Conversely, muscle CPT1 requires more carnitine.

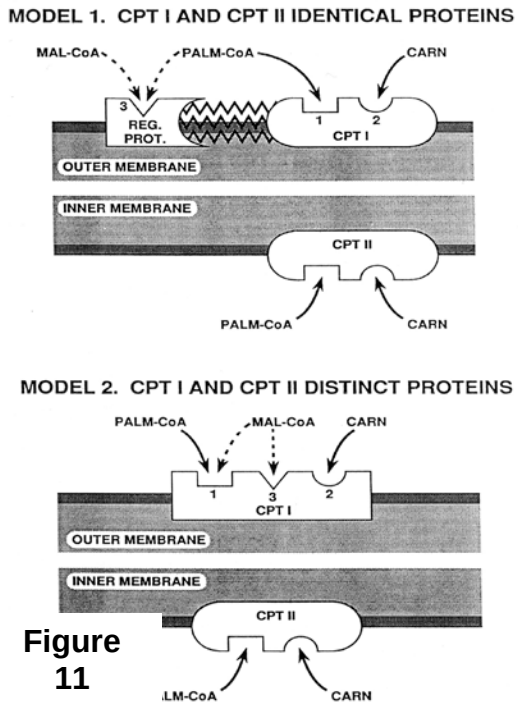
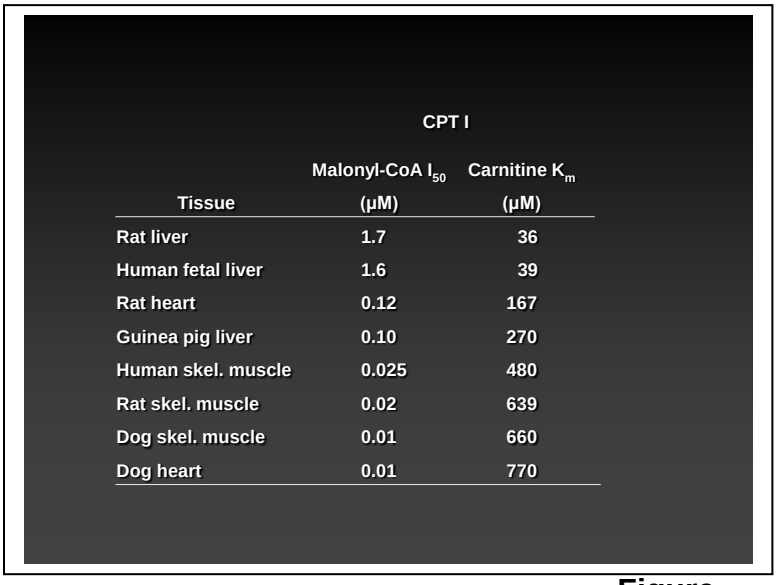



**Figure 11**



CPT I

| Tissue | Malonyl-CoA $I_{50}$ (μM) | Carnitine $K_m$ (μM) |
|---|---|---|
| Rat liver | 1.7 | 36 |
| Human fetal liver | 1.6 | 39 |
| Rat heart | 0.12 | 167 |
| Guinea pig liver | 0.10 | 270 |
| Human skel. muscle | 0.025 | 480 |
| Rat skel. muscle | 0.02 | 639 |
| Dog skel. muscle | 0.01 | 660 |
| Dog heart | 0.01 | 770 |

**Figure 12**

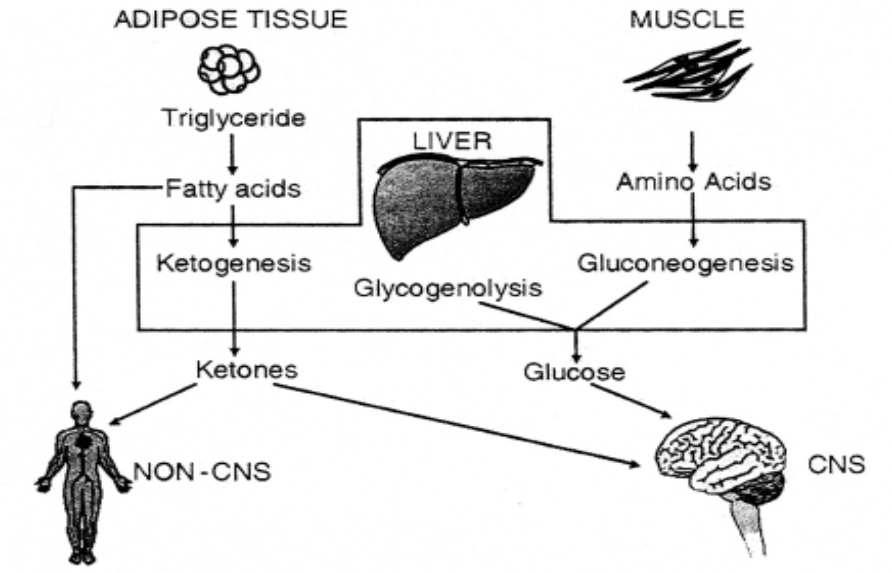
CPT system enzymes gene locations are:

CPT 1A (liver) 11 q 13.1
CPT1B (muscle) 22 q 13.3
CPT 1C (brain, testes) 19 q 13.33
CPT2 (all tissues) 1 p 32
CCAT (all tissues) 3 p 21.31 (CoA: carnitine acyltransferase)

When malonyl-CoA levels drop in fasting or type 1 diabetes, CPT1 becomes active and initiates fatty acid oxidation and ketogenesis.

7. Clinical implications in life and disease
   In the postprandial state the body is in an anabolic state and makes glycogen in the liver, fat synthesis in liver and adipocytes and protein in muscle.

**Figure 13**




In the fasting state, the body becomes catabolic and reverses the metabolism. Glycogen is converted to glucose, free fatty acids are oxidized in all tissues except the brain and the liver produces ketones(5), not $CO_2$ and water. Protein releases alanine and glutamine for gluconeogenesis in the liver and kidney. The brain can oxidize glucose but the liver cannot produce enough glucose to protect it. What saves the brain is the ketones, acetoacetate and β-hydroxybutyrate.

8. Fundamental theses
   1. If too little fat is oxidized, life is threatened.
   2. If too much fat is oxidized, life is threatened.

   A classic example of too little fat oxidation is called systemic carnitine deficiency. A classic example of too much oxidation is diabetic ketoacidosis.

Systemic Carnitine Deficiency

An 11 year-old boy was admitted to Children's Medical Center for recurrent hypoglycemia characterized by episodes of nausea, vomiting, lethargy, confusion or coma. In his last admission he had severe hypoglycemia from 12.5 to 28 mg/dl. AST was186 IU/ml, and ALT was 225 IU/ml. Ammonia was 192 mg/dl. He had an unexpected cardiorespiratory arrest. At post-mortem carnitine was 10-50% of normal in skeletal muscle, heart and liver. All tissues were filled with triglycerides. All other subjects had similar multiple problems.

A summary of the findings:
1. Fasting hypoglycemia
2. No or limited fasting ketones

3. Elevated plasma NH3
4. Hepatic encephalopathy with “flap” and seizures
5. Multiorgan triglyceride storage
6. Muscle weakness and rhabdomyolysis
7. Progressive cardiomyopathy
8. Carnitine low in plasma and tissues
9. Gene defect: mutated carnitine transporter (OCTN2)

The carnitine/cation transporters are three. $OCTN_1$ ($SLC22A_4$), $OCTN_2$ ($SLC22A_5$), AND $OCTN_3$ ($SLC22A_{21}$). With the deficiency of carnitine, patients have rhabdolyolysis, cardiomyopathy, and hepatic encephalopathy. All the tissues have excess triglycerides. Patients should never be allowed to fast. They must take in large daily amounts of carnitine. They should be taken to the hospital in the face of any illness and infused instantly with glucose to protect against hypoglycemia.

**Figure 16**
**A case of ketoacidosis**

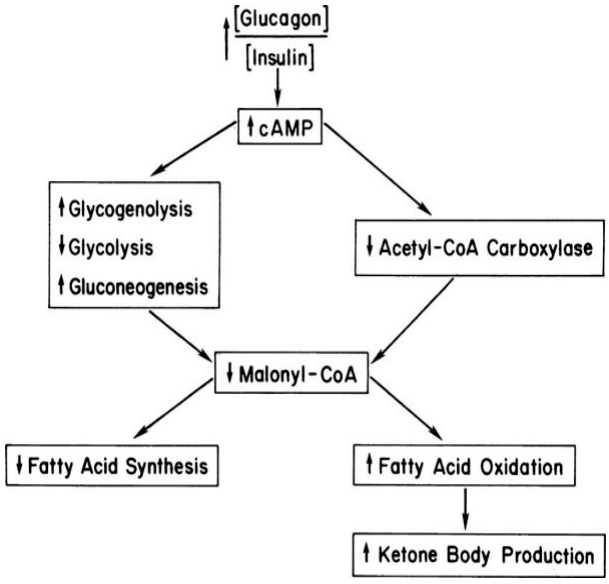



**Figure 17**

**Table 46-1.** Hormone Values in Patients With Diabetic Ketoacidosis

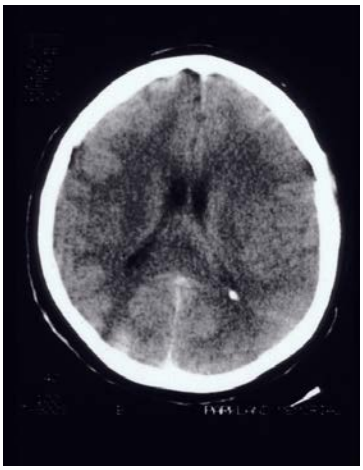
| Hormone | Controls | Patients |
|---|---|---|
| Insulin (μU/mL) | 15 ± 2 | — |
| C peptide (ng/mL) | 2.4 ± 0.07 | — |
| Glucagon (pg/mL) | 99 ± 19 | 741 ± 247 |
| Epinephrine (ng/mL) | 0.05 ± 0.03 | 2.6 ± 1.3 |
| Norepinephrine (ng/mL) | 0.2 ± 0.08 | 3.8 ± 1.1 |
| Cortisol (μg/dL) | 10.5 ± 2 | 50.4 ± 4.9 |
| Growth hormone (ng/mL) | 0.7 ± 0.1 | 4.6 ± 1.6 |
| Renin (GU × $10^4$/mL) | 0.3 ± 0.1 | 13.2 ± 4.6 |
| Aldosterone (ng/dL) | 7.8 ± 2.3 | 83 ± 25 |
| Pancreatic polypeptide (pg/mL) | 93 ± 11 | 691 ± 200 |

Data from Waldhäusl W, Kleinberger G, Korn A et al: Severe hyperglycemia: effects of rehydration on endocrine derangements and blood glucose concentration. Diabetes 28:577-584, 1979.
GU, Goldblatt Unit.
Control values were obtained in the basal state after an overnight fast. Plasma renin concentration and aldosterone were measured in subjects consuming 120 mmol $Na^+$ per day. Data are means ± SEM.

W.G. was a 21 year-old black male with insulin-dependent diabetes mellitus who was admitted in diabetic acidosis. Admission hemoglobin was 20.3 g/dl, hematocrit was 60.8%, WBC was 21,200, lactate 2.6 mM, glucose 416 mg/dl, bicarbonate 6.3mM, amylase 182 (normal <110), lipase 798 (normal <208), pH 7.15, pO2 99 mM. He responded to treatment but then developed a massive stroke due to hyperglycemia and overproduction of plasminogen activator inhibitor type 1. He died from his stroke. The pathophysiology is shown in *Figures 16, 17*

**CEREBRAL THROMBOSIS IN DKA**

**W.G., a 21 y/o BM with known insulin-dependent diabetes mellitus, was admitted in diabetic ketoacidosis. Admission hemoglobin 20.3 g/dl, hematocrit 60.8%, WBC 21,200. Ethanol negative. Trace salicylate. Lactate 2.0 mM, amylase 182 (nl <110), lipase 798 (nl <208), pH 7.15, $pO_2$ 99 mm.**



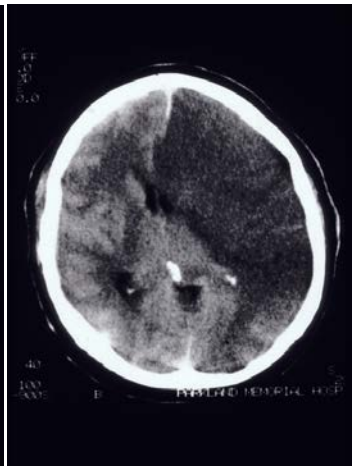


**Figure 18** **Figure 19** **Figure 20**

Fat is crucial. If you can't oxidize it you can die. If you oxidize too much you may also die. These are metabolic crises.

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