Appendix C – Detailed Research on HCA

Section I : Basic Mechanisms of Action

Overview

Whenever the rate of glycolysis exceeds energy requirements and/or the capacity to produce and store glycogen, the resulting acetyl-CoA units derived from carbohydrates (and under some conditions, also proteins) are turned into fatty acids and cholesterol at the first step of the Krebs Cycle. Acetyl-CoA, the primary substrate for fatty acid biosynthesis, is a product of pyruvate oxidation within the mitochondrion when demands for energy in the form of ATP are low. However, fatty acid biosynthesis does not take place within the mitochondrion, but instead occurs within the cytosol of the cell, and acetyl-CoA cannot cross the mitochondrial membrane to accomplish this synthesis. Acetyl-CoA can cross the membrane only as citrate, and therefore the excess acetyl-CoA within the mitochondrion is exported in this form. In the cytosol, ATP-citrate lyase is required to cleave citrate in the above reaction to release acetyl-CoA. *HCA works by acting as a competitive inhibitor of the enzyme ATP-citrate lyase* within the following reaction pathway of fatty acid biosynthesis in the presence of the divalent ion of magnesium:

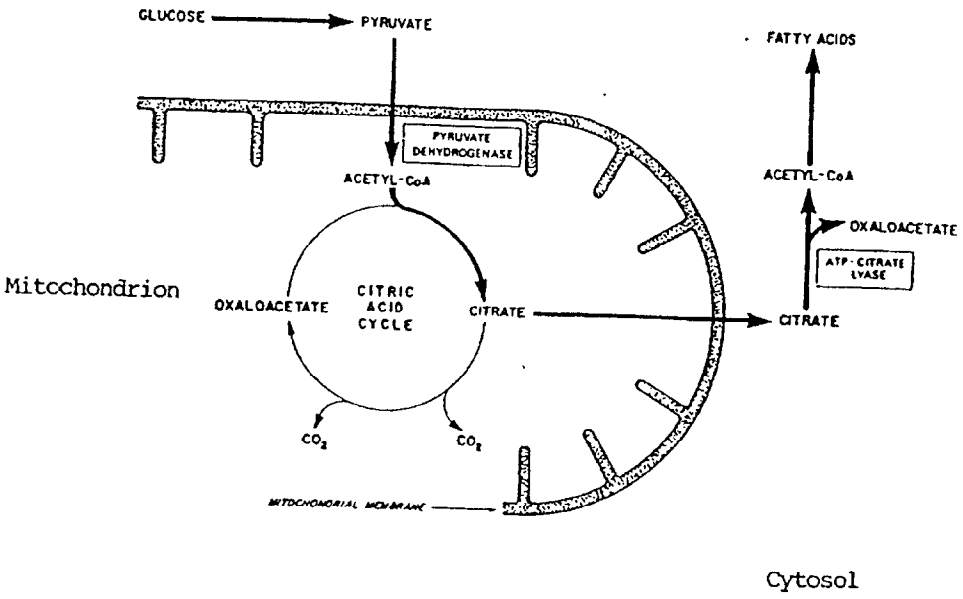
$$\underset{\text{[in the mitochondrion]}}{\text{ATP + citrate + CoA}} \longrightarrow \underset{\text{[outside the mitochondrion in the cytosol]}}{\text{acetyl-CoA + oxaloacetate + ADP + } P_i}$$

where ATP = adenosine triphosphate, CoA = coenzyme A, ADP = adenosine diphosphate and P_i = orthophosphate ion. Citrate is synthesized from oxaloacetate and acetate.

The full effects of HCA reflect the extensive role of citrate in the metabolism of carbohydrates and the synthesis of lipids. Citrate does more than merely ferry acetyl units from the mitochondrion into the cytosol. Once in the cytosol, citrate itself stimulates the fatty acid biosynthetic pathway through its activation of acetyl-CoA carboxylase. At the same time, it also serves to regulate glycolysis at three points. Citrate inhibits both phosphofructokinase and pyruvate dehydrogenase. Likewise, the presence of excess citrate at the point at which acetyl-CoA enters the Krebs Cycle inhibits the enzyme citrate synthase from producing additional citrate.

The effects of HCA within this framework are complex. Through its basic action of inhibiting ATP-citrate lyase, HCA causes an increase in the extramitochondrial pool of citrate, and thereby also in an increase in the inhibition of glycolysis at the regulatory junctures. HCA itself may mimic some of the regulatory activities of citrate. Three secondary effects thus appear with the administration of HCA. First, the production of fatty acids and cholesterol is reduced under most conditions. Second, the glycolytic pathway is slowed. Third, carbon units are redirected toward glycogen production. The following figures illustrate these points.

The Krebs/Citric Acid Cycle in Fatty Acid Synthesis from Glucose



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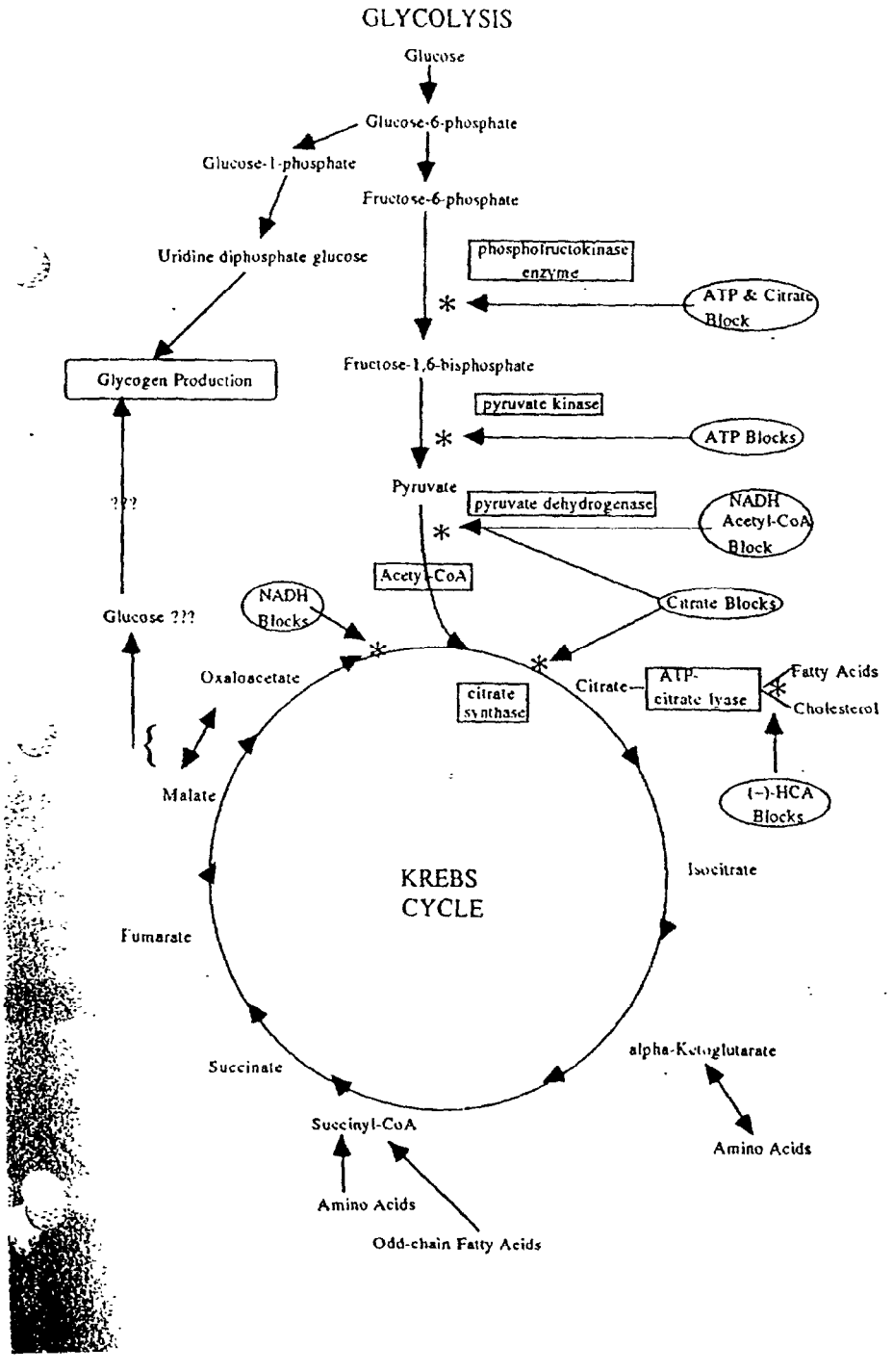
Partial Regulation of Glycolysis (Metabolism of Carbohydrates) and the Krebs Cycle by HCA



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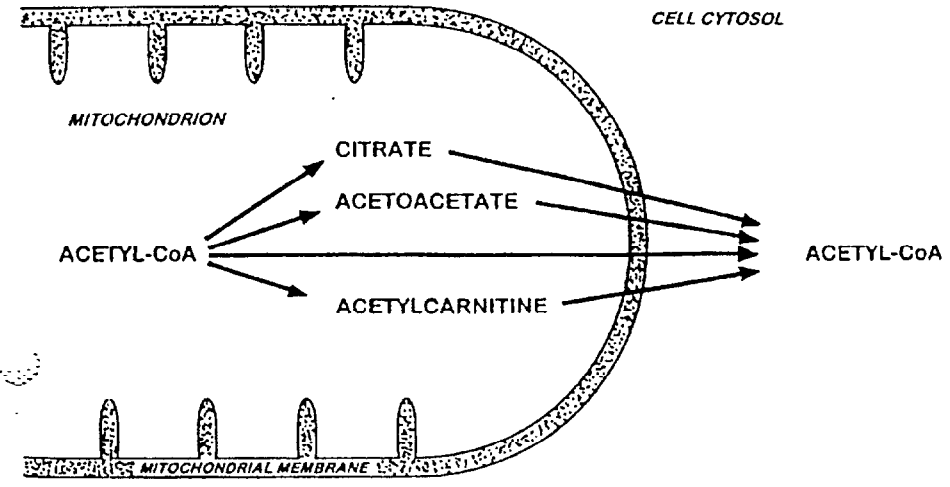
Possible Pathways of Acetyl-CoA Across the Mitochondrial Membrane



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HCA Inhibits ATP-Citrate Lyase

In Vitro Study:

"...a relatively powerful inhibition of citrate cleavage is observed in the presence of (–)-hydroxycitrate. The stereoselectivity of the inhibition of citrate cleavage by hydroxycitrates... shows that (–)-hydroxycitrate is a much more powerful inhibitor than (+)-*allo*-hydroxycitrate."

Watson, John A., Marie Fang, and John M. Lowenstein (1969). Tricarballylate and hydroxycitrate: Substrate and inhibitor of ATP:citrate oxaloacetate lyase. **Archives of Biochemistry and Biophysics** 135 (1969) 209-217.

In Vitro Study:

"There are three citrate enzymes which catalyze the same bond-making and -breaking reaction which involves the equilibrium of citrate with oxalacetate and an acetyl moiety.... (4*S*)-OHcit-(pn_{cit}) [(–)hydroxycitrate] is a very potent linear competitive inhibitor of ATP-citrate lyase from rat liver....the other stereoisomer with a [*sic*] hydroxyl group substituted on carbon 4, was a less effective inhibitor, but a more potent inhibitor than the two isomers with hydroxyl groups substituted on carbon 2....The four hydroxycitrates bind well and all undergo a reaction except (4*S*)-OHcit-(pn_{cit}) [(–)hydroxycitrate] which is found to be an extremely potent binder."

Sullivan, Ann C., Manoranjan Singh, Paul A. Srere, and Jenny P. Glusker (1977c). Reactivity and inhibitor potential of hydroxicitrate isomers with citrate synthase, citrate lyase, and ATP citrate lyase. **The Journal of Biological Chemistry** 252, 21 (November 10, 1977) 7583-7590.

Review Article:

"In the breakdown of foodstuffs, pyruvate derived from carbohydrate and fatty acids derived from fat are converted to acetyl-CoA by the intramitochondrial enzyme systems. Under normal conditions of carbohydrate utilization, the rate of oxidation of the acetyl group of acetyl-CoA via the citric acid cycle is determined by the energy demands of tissue, or its equivalent, the availability of ADP. When the carbohydrate intake of an animal is in excess of its energy requirements the glycogen stores become filled. Thereafter excess carbohydrate is broken down to pyruvate. The reactions of glycolysis occur in the extramitochondrial space of the cell. However, oxidation of pyruvate to acetyl-CoA occurs in the mitochondria. Acetyl groups not required for energy production are converted into fatty acids. In the rat, fatty acid synthesis occurs predominantly in the extramitochondrial space of the cell [i.e., the cytosol]. The transfer of the acetyl group of acetyl-CoA from the intramitochondrial space into the cytoplasm is thus an important step in the conversion of carbohydrate into fat by non-ruminant mammals."

Lowenstein, John M. (1970). Experiments with (–)-hydroxycitrate. In W. Bartley, H.L. Kornberg and J.R. Quayle, eds., **Essays in Cell Metabolism** (New York: Wiley—Interscience, 1970) 153-166.

[redacted]

HCA Does Not Inhibit Synthesis from Acetate, a Minor Pathway

In Vitro **Study:**

"In isolated hepatocytes it [HCA] inhibits fatty acid synthesis from glucose, but it does not affect fatty acid synthesis from acetate."

Beynen, A.C. and M.J.H. Geelen (1982). Effects of insulin and glucagon on fatty acid synthesis from acetate by hepatocytes incubated with (–)-hydroxycitrate. **Endokrinologie** 79, 2 (1982) 308-310.

Perfusion Experiment:

"In this paper an inhibition of hepatic cholesterol and fatty acid synthesis by (–)hydroxycitrate in the isolated perfused rat liver is reported; acetate reversed the effect on fatty acid synthesis."

Barth, C., J. Hackenschmidt, H. Ullmann, and K. Decker (1972). Inhibition of cholesterol synthesis by (–)-hydroxycitrate in perfused rat liver. Evidence for an extramitochondrial mevalonate synthesis from acetyl coenzyme A. **FEBS Letters** 22, 3 (May 1972) 343-346.

Perfusion Experiment:

"In the presence of (–)-hydroxycitrate, translocation of acetyl units via systems other than citrate or acetoacetate contributes 78 and 20% of the total carbon incorporated into fatty acids and sterols, respectively....It follows that the pathways not involving citrate or acetoacetate could contribute only 15 and 5% of the supply of carbon for fatty acid and sterol synthesis. Our data confirm that the citrate cleavage pathway is the predominant form of acetyl translocation in rat liver."

Endemann, Gerda, Patrick G. Goetz, John Edmond and Henri Brunengraber (1982). Lipogenesis from ketone bodies in the isolated perfused rat liver. **The Journal of Biological Chemistry** 257, 7 (1982) 3434-3440.

Articles

1. M. G. Soni, G.A. Burdock, H.G. Preuss, S.J. Stohs, S. E. Ohia, D. Bagchi: **Safety assessment of (-)-hydroxycitric acid and Super CitriMax, a novel calcium/potassium salt.** Food and Chemical Toxicology 42 (2004) 1513-1529

Watson, John A., Marie Fang, and John M. Lowenstein (1969). **Tricarballylateand hydroxycitrate: Substrate and inhibitor of ATP: citrate oxaloacetate lyase.** Archives of Biochemistry and Biophysics 135, 1(1969) 209-217

Watson, John A. and John M. Lowenstein (1970). **Citrate and conversion of carbohydrate into fat.** The Journal of Biological Chemistry 245, 22 (1970) 5993-6002.

Lowenstein John M. (1970) **Experiments with (-)-hydroxycitrate.** In W Bartley, H.L. Kornberg and J.R. Quayle, eds., Essays in Cell Metabolism (New York: Wiley Interscience, 1970) 153-166

Sullivan, Ann C., Manoranjan Singh, Paul A. Srere, and Jenny P Glusker (1977c).
Reactivity and inhibitor potential of hydroxycitrate isomers with citrate synthase, citrate lyase, and ATP citrate lyase. The Journal of Biological Chemistry 252, 21 (November 10, 1977) 7583-7590.