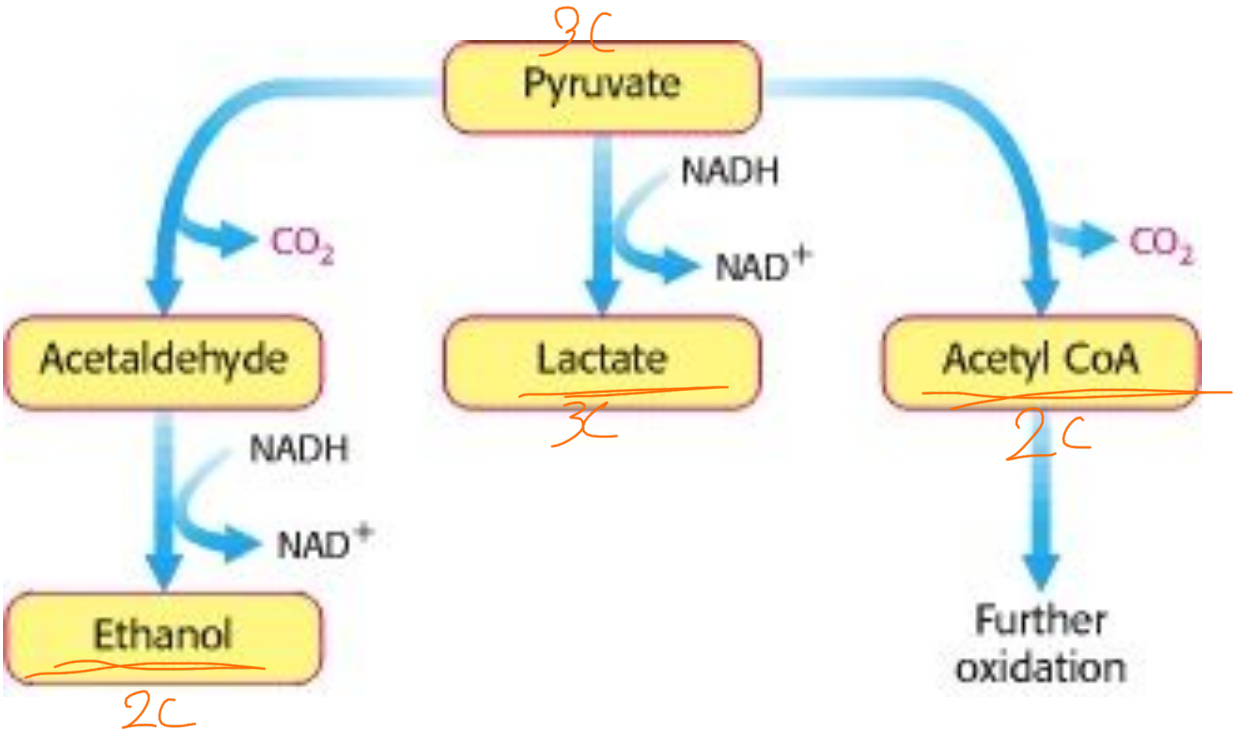
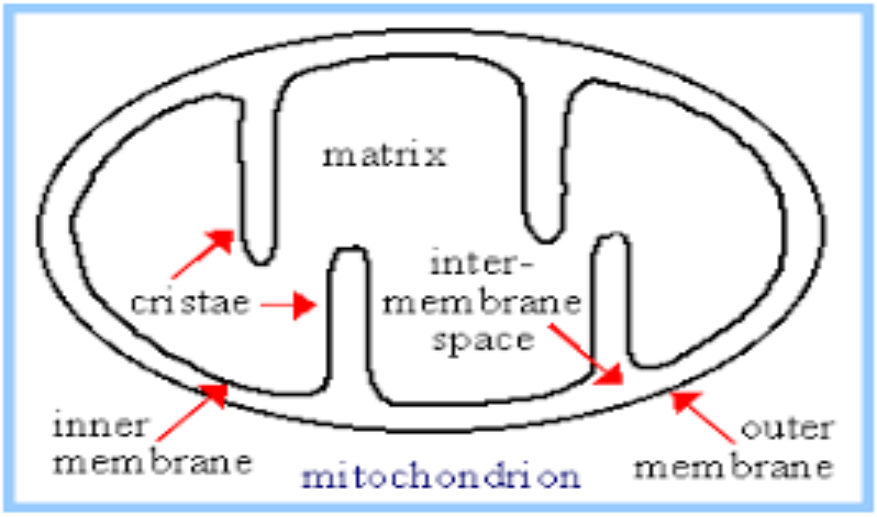
Pyruvate metabolism

Chapter 3



- Before entering the citric acid cycle, the carbon skeletons of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA
- **pyruvate dehydrogenase (PDH) complex**
- located in the mitochondria of eukaryotic cells



Inner membrane foldings called Cristae contain ETC

The matrix contains Pyruvate dehydrogenase enzymes, and enzymes of the Kreb cycle

- Five cofactors, four derived from vitamins, participate in the reaction mechanism
- combination of covalent modification and allosteric regulation
- PDH complex consists of multiple copies of three enzymes:
  - **Pyruvate dehydrogenase (E1)**
  - **Dihydrolipoamide transacetylase (E2)**
  - **Dihydrolipoamide dehydrogenase (E3)**
- Also part of the complex are two regulatory enzymes
  - A protein kinase
  - A phosphoprotein phosphatase

The pyruvate dehydrogenase reaction involves multiple coenzymes

Coenzyme	Subunit	Role in catalysis
thiamine pyrophosphate	E_1	provides a carbanion for nucleophilic attack on the substrate
lipoamide	E_2	transfers substrate to coenzyme A, retains hydrogen
flavin adenine dinucleotide (FAD)	E_3	transfers H_2 from lipoamide to NAD^+

Step 1: E1

TPP + Pyruvate → Hydroxyethyl TPP + CO_2

Step 2:

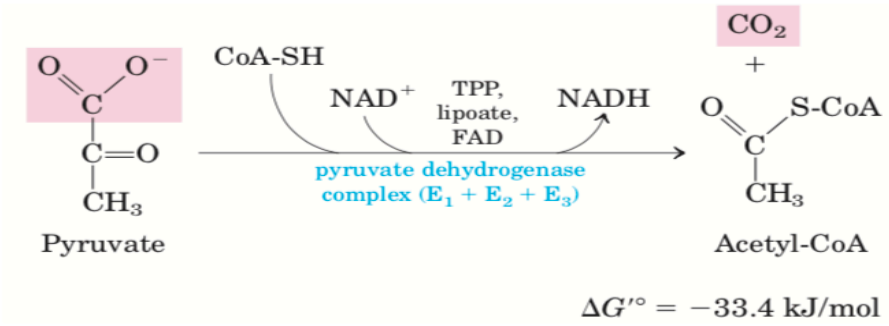
Hydroxyethyl TPP + lipoamide → TPP + Acetyl lipoamide

Step 3: E2

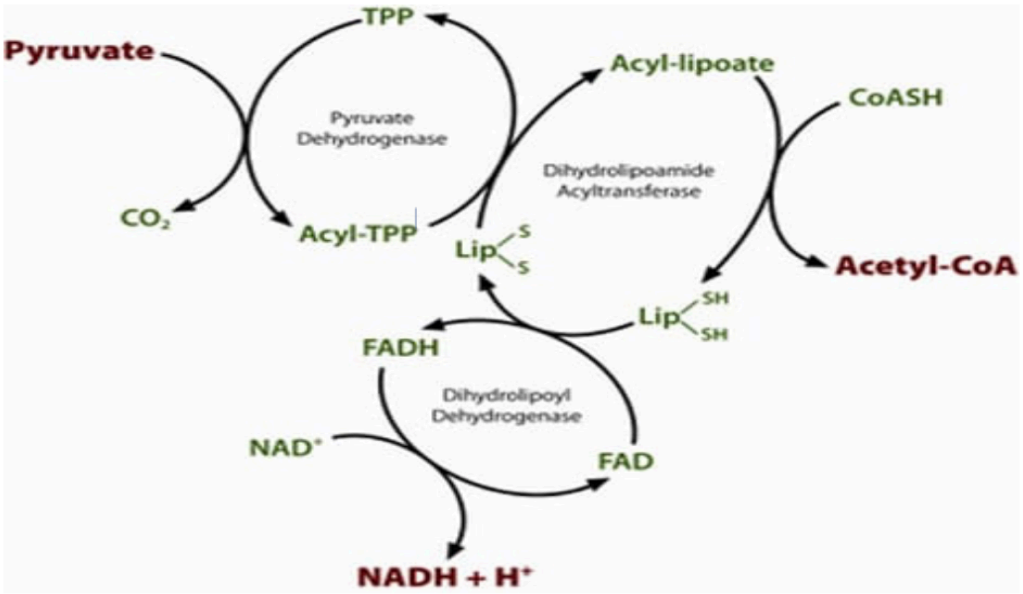
CoA-SH + Acetyl lipoamide → Acetyl CoA + SH-SH-E_2

step4: E_3 -SH-SH + FAD → lipoamide + $FADH_2$ + NAD+ → NADH + FAD + H^+

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation,** an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO2



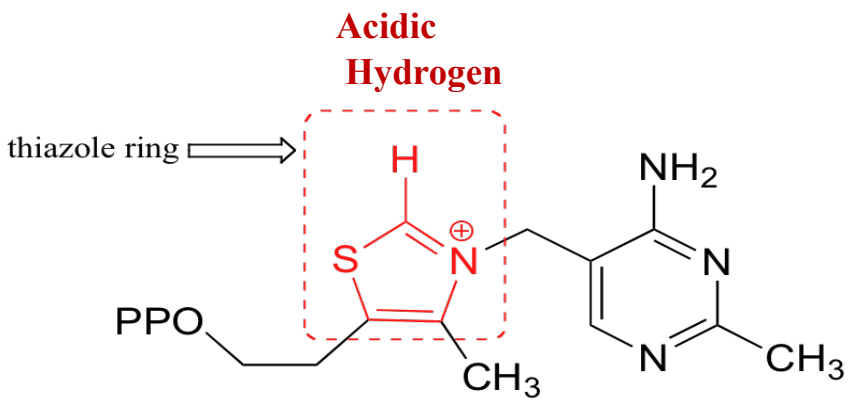
Pyruvate + CoA-SH + NAD+ → CO2 + acetyl-CoA + NADH + H+



PDH Complex five different coenzymes or prosthetic groups

- Thiamine pyrophosphate (TPP) Thiamine (B1)
- Flavin adenine dinucleotide (FAD) Riboflavin (B2)
- Coenzyme A (CoA, sometimes de- noted CoA-SH, to emphasize the role of the OSH group pantothenate (B5)
- Nicotinamide adenine dinucleotide (NAD) Niacin (B3)
- Lipoamide

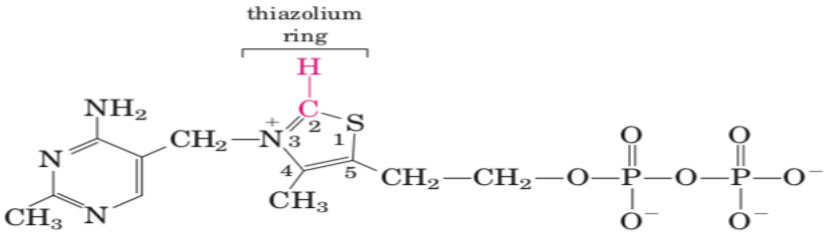
Thiamine pyrophosphate



thiamine diphosphate (TPP)

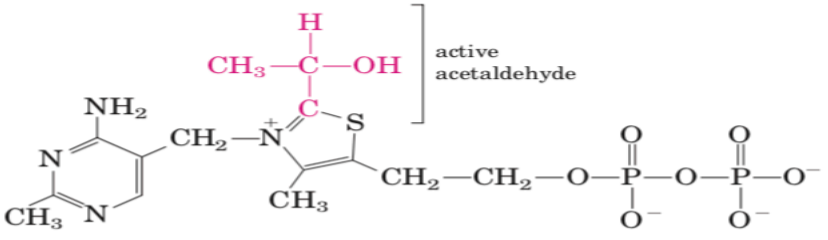
The carbanion then acts as a strong nucleophile **carbanion** Initiates a nucleophilic attack on the carbonyl **carbon** of pyruvate

1. The keto C of pyruvate reacts with the carbanion of TPP on E1 to yield an addition compound. The electron-pulling (+) charged N of the thiazole ring promotes CO2 loss. Hydroxyethyl-TPP remains.
2. The hydroxyethyl carbanion on TPP of E1 reacts with the disulfide of lipoamide on E2. What was the keto C of pyruvate is oxidized to a carboxylic acid, as the lipoamide disulfide is reduced to a dithiol
3. Acetate is transferred from the thiol of lipoamide to the thiol of coenzyme A, yielding acetyl CoA.
4. Dihydrolipoamide is reoxidized to the disulfide as 2 e- + 2 H+ are transferred to FAD.
5. The resulting FADH2 is reoxidized by electron transfer to NAD+, to yield NADH + H+.



Thiamine pyrophosphate (TPP)

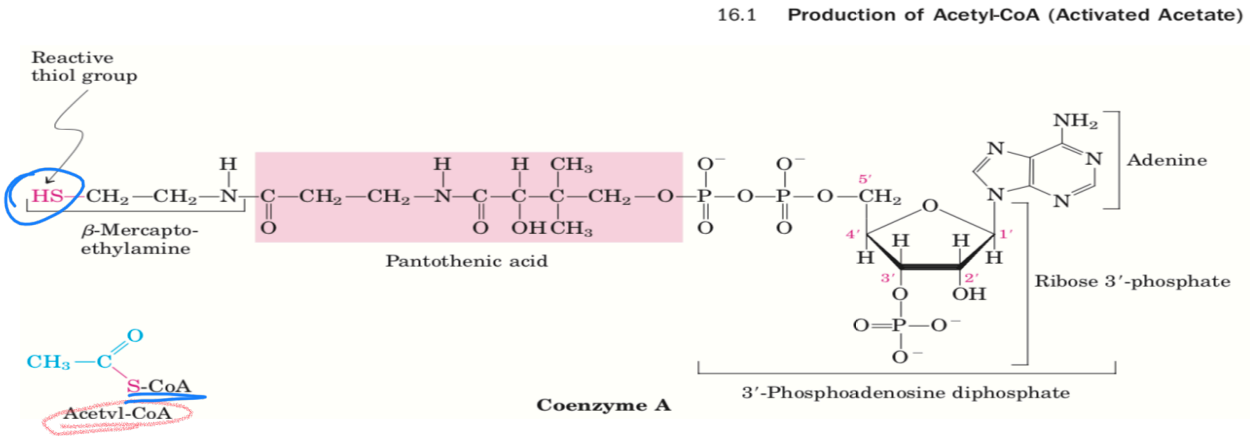
(a)



Hydroxyethyl thiamine pyrophosphate

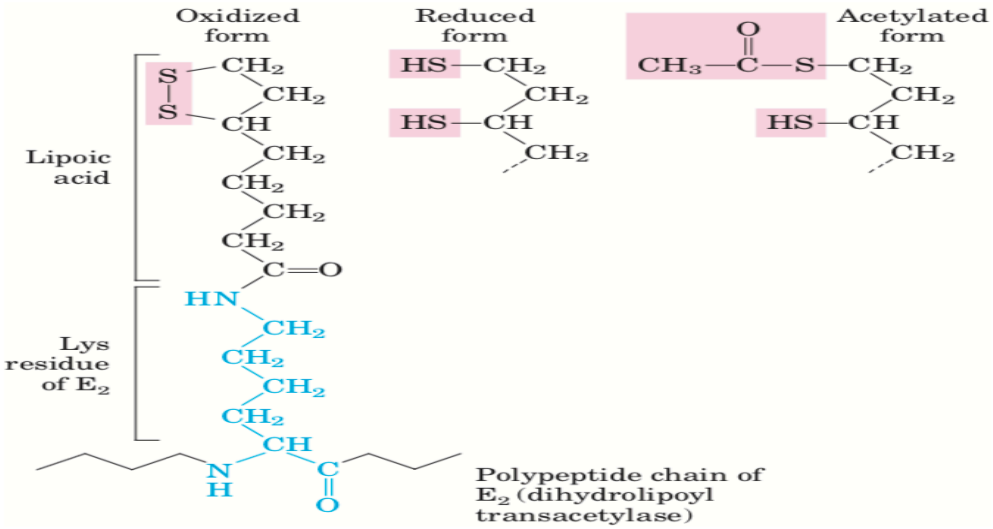
Coenzyme A

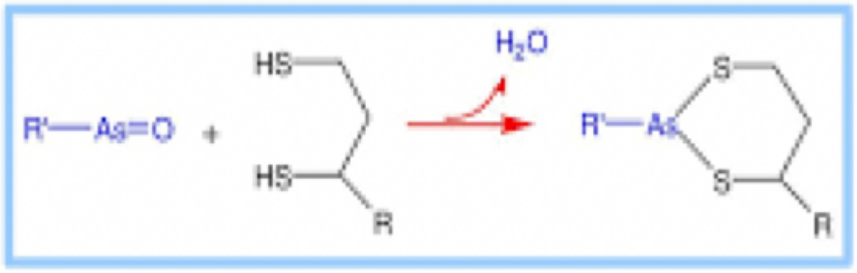
- Coenzyme A -has a reactive thiol (OSH)
- Role of CoA as an acyl carrier- Acyl groups are covalently linked to the thiol group, forming **thioesters**



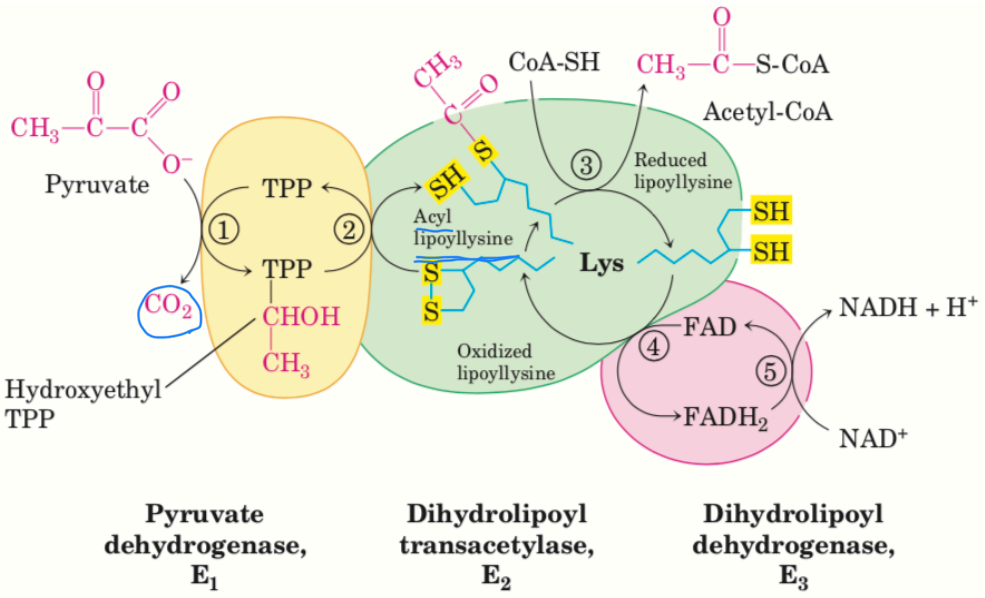
lipoate

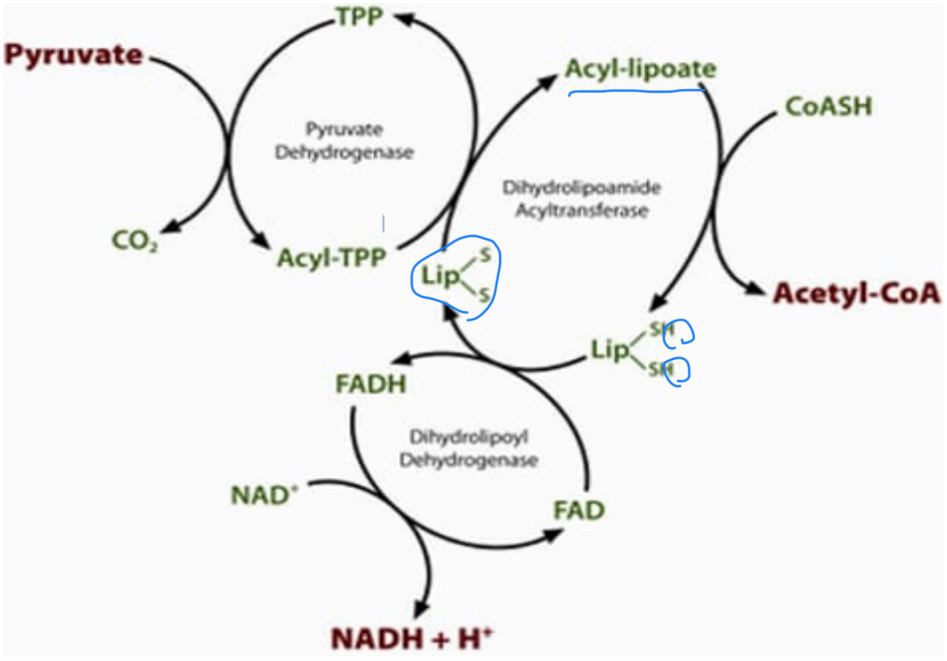
lipoate has two thiol groups that can undergo reversible oxidation to a disulfide bond (--S—S--)





Organic arsenicals are potent inhibitors of lipoamide-containing enzymes such as Pyruvate Dehydrogenase. These highly toxic compounds react with dithiols such as the functional group of lipoate.





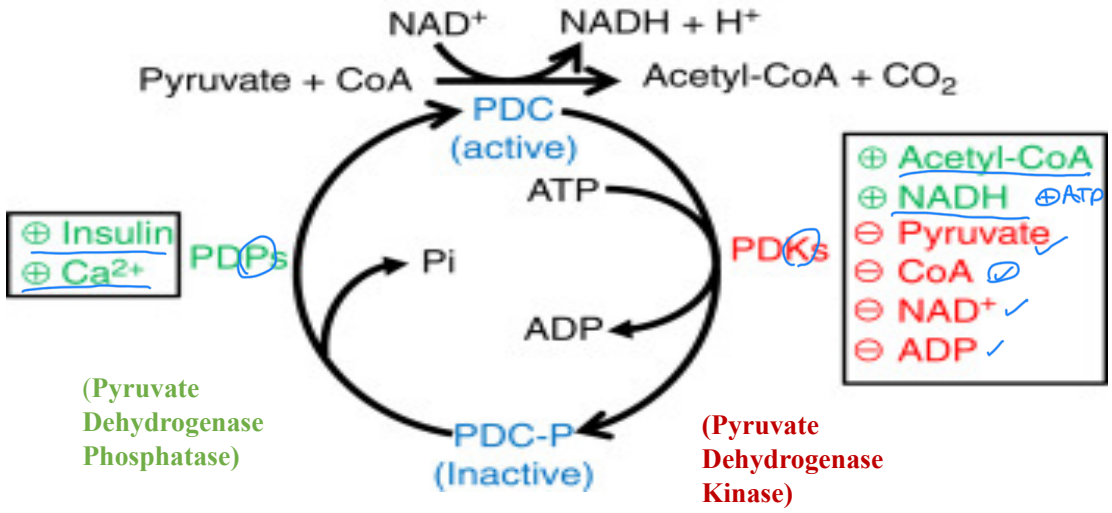
- FAD is a prosthetic group, permanently part of E3.Reaction:
- FAD + 2 e- + 2 H+ □FADH2

Regulation of Pyruvate Dehydrogenase Complex:

Product inhibition by NADH & acetyl CoA:

NADH competes with NAD+ for binding to E3. Acetyl CoA competes with CoA for binding to E2.

- Specific regulatory Kinases & Phosphatases associated with Pyruvate Dehydrogenase in the mitochondrial matrix:
  - Pyruvate Dehydrogenase Kinases catalyze phosphorylation of serine residues of E1, inhibiting the complex.
  - Pyruvate Dehydrogenase Phosphatases reverse this inhibition.



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pyruvate-dehydrogenase-phosphatase

Regulation of PDH activity

- PDH activity is inhibited by reversible phosphorylation of the E1
- The phosphorylation □PDH kinase
- Dephosphorylation to restore PDH activity phosphatases

PDH Kinase (a special regulatory enzyme which is part of the PDH multienzyme complex)

The PDH kinase enzyme is activated by NADH and acetyl-CoA and inhibited by ADP, NAD+ and by free coenzyme A

During starvation

- Pyruvate Dehydrogenase Kinase increases in amount in most tissues, including skeletal muscle, via increased gene transcription.
- Under the same conditions, the amount of Pyruvate Dehydrogenase Phosphatase decreases
- The resulting inhibition of Pyruvate Dehydrogenase prevents muscle and other tissues from catabolizing glucose & gluconeogenesis precursors.
- Metabolism shifts toward fat utilization.
- Available glucose is spared for use by the brain.