ENZYMES:

LACTATE DEHYDROGENASE AND CREATINE KINASE

LACTATE DEHYDROGENASE (LD)

- Tetramer (4 polypeptide chains) bound together by disulfide bonds; two possible subunit types, H and M
- Inhibitors include reagents with reactivity against thiol groups such as Hg²⁺ (inhibition can be reversed by the addition of cysteine or glutathione), borate, oxalate, EDTA, pyruvate and lactate
LACTATE DEHYDROGENASE (LD)

FIVE ISOENZYMES FOUND IN SERUM

LD-1: $H_4$  \hspace{1cm} PREDOMINATE IN HEART AND RBC
LD-2: $H_3M$  \hspace{1cm} PREDOMINATE IN SPLEEN, LUNGS, PLATELETS, AND LYMPH NODES
LD-3: $H_2M_2$  \hspace{1cm} PREDOMINATE IN LIVER AND SKELETAL MUSCLE
LD-4: $HM_3$  \hspace{1cm} PREDOMINATE IN LIVER AND SKELETAL MUSCLE
LD-5: $M_4$  \hspace{1cm} ALSO FOUND IN KIDNEY, BRAIN, PANCREAS

LACTATE DEHYDROGENASE (LD)

CLINICAL SIGNIFICANCE

- SERUM LD ACTIVITY IS INCREASED IN:
  - HEART DISEASES - MYOCARDIAL INFARCT, MYOCARDITIS, CARDIAC FAILURE (LD-1 & LD-2)
  - LIVER DISEASES - HEPATITIS, CIRRHOSIS, OBSTRUCTIVE JAUNDICE (LD-5 AND SOME LD-4)
  - MUSCLE DISEASES - MUSCULAR DYSTROPHY, INJURY (LD-5)
  - HEMOLYTIC DISORDERS - MEGALOBLASTIC ANEMIA, HEMOLYTIC ANEMIA (LD-1 & LD-2)
LACTATE DEHYDROGENASE (LD)
CLINICAL SIGNIFICANCE (CONT’D)

• SERUM LD ACTIVITY IS INCREASED IN:
  
PULMONARY DISEASES – PULMONARY EMBOLISM, EXTENSIVE PULMONARY PNEUMONIA (LD-3)
  
CARCINOMAS – HODGKIN’S, ABDOMINAL AND LUNG CANCERS, LIVER METASTASES (LD-4 & LD-5); LD-1 MAY BE INCREASED IN SOME GERM CELL TUMORS

APPEARANCE OF LD-6 REFLECTS LIVER INJURY SECONDARY TO SEVERE CIRCULATORY INSUFFICIENCY - POOR PROGNOSIS

LD: MEASUREMENT

SAMPLE

• SERUM IS PREFERRED; PLASMA MAY BE CONTAMINATED WITH PLATELETS
  
• HEMOLYSIS FALSELY ELVATES LD ACTIVITY DUE TO ERYTHROCYTE LD (100-150X THAT OF SERUM)
  
• LD-4 AND LD-5 ESPECIALLY LABILE TO COLD; RECOMMEND STORAGE OF SPECIMEN AT ROOM TEMPERATURE - STABLE FOR UP TO 2 DAYS IF REMOVED FROM CELLS

LD VALUES HIGHEST IN INFANTS (5X ADULT) AND GRADUALLY DECREASE TO ADULT RANGE
**LD: MEASUREMENT**

- LD activity may be measured in either direction;
  - Forward: lactate → pyruvate
  - Reverse: pyruvate → lactate

- Both substrates may inhibit the enzyme at very high concentrations

- Reverse reaction faster; but substrate inhibition greater

- Lactate and NAD are less inhibitory, measure increasing absorbance – forward reaction recommended

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**LD: MEASUREMENT**

**ISOENZYME FRACTIONATION**

- Substrate selectivity: LD-1 will reduce 2-oxybutyrate; less activity with other LD isoenzymes

- Immunoprecipitation: use anti-M subunit antibody to precipitate all enzymes except LD-1

- Isoenzyme electrophoresis
ENZYMES: LACTATE DEHYDROGENASE AND CREATINE KINASE

LD: MEASUREMENT

ISOENZYME ELECTROPHORESIS

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD-1</td>
<td>14-26%</td>
</tr>
<tr>
<td>LD-2</td>
<td>29-39%</td>
</tr>
<tr>
<td>LD-3</td>
<td>20-26%</td>
</tr>
<tr>
<td>LD-4</td>
<td>8-16%</td>
</tr>
<tr>
<td>LD-5</td>
<td>6-16%</td>
</tr>
</tbody>
</table>

NORMAL PATTERN

AMI $\rightarrow$ PULMONARY EMBOLISM

MEGALOBLASTIC ANEMIA $\rightarrow$ LIVER DISEASE

HEMOLYSIS

CREATINE KINASE (CK)

CATALYZES THE REVERSIBLE TRANSFER OF A PHOSPHATE GROUP FROM ATP TO CREATINE
CREATINE KINASE (CK)

- Requires the activator Mg²⁺ however excess Mg²⁺ is inhibitory
- Other inhibitors include Mn²⁺, Ca²⁺, Zn²⁺, Cu²⁺, sulfhydryl binding reagents, citrate, fluoride, uric acid, excess ATP, etc.
- Dimer (2 peptide chain - B and M joined by disulfide bonds)
- Three isoenzymes CK-1 BB
  CK-2 MB
  CK-3 MM
- CK relatively unstable - activity lost when - SH group oxidized. Activity can be partially restored by addition of sulfhydryl compounds such as N-acetylcysteine (NAC), monothioglycerol, dithioerythritol, or dithiothreitol (Cleland's reagent)

CREATINE KINASE (CK)

- Tissue source
  CK-1 (BB) Brain, nerve tissue and small amount in prostate, intestine, lung, bladder, kidney, uterus, thyroid,
  CK-2 (MB) Heart muscle, skeletal muscle
  CK-3 (MM) Skeletal and heart muscle
CREATINE KINASE (CK)

• ISOFORMS (SUBTYPES OF THE ISOENZYMES) ARISE FROM REMOVAL OF THE TERMINAL LYSINE ON THE M PEPTIDE CHAIN OF THE ISOENZYMES RELEASED FROM TISSUES

• CARBOXYPEPTIDASES FOUND IN BLOOD CATALYZE THIS REMOVAL

CK-MM Isoforms

CK-MB Isoforms

CREATINE KINASE (CK)

CK ISOFORMS

RELATIVE AMOUNTS OF CK ISOFORMS INDICATE HOW LONG CK HAS BEEN IN THE CIRCULATION AFTER RELEASE FROM TISSUE

HIGH ACTIVITIES OF THE UNMODIFIED ISOFORMS INDICATE PRESENCE OF AN ACUTE INJURY
CREATINE KINASE (CK)

ATYPICAL CK ISOENZYMES

• MITOCHONDRIAL CK (CK-Mi, CK-Mt) BOUND TO MITOCHONDRIAL MEMBRANES OF MUSCLE, BRAIN AND LIVER

• MIGRATES ELECTROPHORETICALLY TO A POINT CATHODAL TO CK-MM

• OCCURS IN SERUM BOTH AS A DIMER OF IDENTICAL SUBUNITS AND AS AN AGGREGATE OF HIGH MW (SOMETIMES REFERRED TO AS MACRO CK TYPE-2)

• NOT PRESENT IN NORMAL SERUM – ONLY SEEN WHEN THERE IS EXTENSIVE TISSUE DAMAGE - DETECTED IN SEVERELY ILL PATIENTS E.G. ADULTS WITH MALIGNANT TUMORS AND CHILDREN WITH CARDIAC ABNORMALITIES
CREATINE KINASE (CK)

- TOTAL CK ACTIVITY IN SERUM DEPENDS UPON MUSCLE MASS, LEVEL OF PHYSICAL ACTIVITY, RACE, GENDER, AND AGE
- FEMALES AVERAGE 25% LOWER LEVELS THAN MALES
- AFRICAN-AMERICAN MALES AVERAGE 50% HIGHER
- LEVELS ARE HIGHER IN CHILDREN THAN ADULTS
- SERUM CK ACTIVITY IS 3X ADULT LEVELS IN NEONATES DUE TO CK-1 FROM UTERUS AND PLACENTA
- LEVELS ARE LOWER IN GERIATRIC THAN MIDDLE AGED ADULT
- IN HEALTHY ADULTS, >95% SERUM CK ACTIVITY IS CK-3

CREATINE KINASE (CK)

CLINICAL SIGNIFICANCE

CK ELEVATIONS ARE SEEN IN:

1. SKELETAL MUSCLE DISORDERS (PRIMARILY CK-3 ISOENZYME; SOME CK-2)
   - MUSCULAR DYSTROPHY (50-100 X URL SEEN IN DUCHENNE TYPE OF MD; 3-6 X URL SEEN IN 50-80% OF THE FEMALE CARRIERS OF DUCHENNE'S)
   - POLYMYOSITIS
   - VIRAL MYOSITIS
   - HYPOTHYROIDISM
   - MALIGNANT HYPERThERMIA (FAMILIAL CONDITION CHARACTERIZED BY HIGH FEVER FOLLOWING ADMINISTRATION OF INHALATION ANESTHESIA)
   - RHABDOMYOLYSIS
   - MUSCLE TRAUMA

SERUM CK ACTIVITY IS USUALLY NORMAL IN NEUROGENIC MUSCLE DISEASES: I.E. MYASTHENIA GRAVIS, MULTIPLE SCLEROSIS
CREATINE KINASE (CK)

CLINICAL SIGNIFICANCE

CK ELEVATIONS ARE SEEN IN:

II. HEART DISEASE (CK-2 AND CK-3 ELEVATES; CK-2 > 6%)
   - MYOCARDIAL INFARCTION (CK BEGINS TO RISE IN 4-8 HOURS,
     PEAKS IN APPROXIMATELY 24 HOURS, TOTAL CK RETURNS TO
     NORMAL IN 3-4 DAYS; CK-2 RETURNS TO NORMAL IN 2-3 DAYS;
     CK-3 ISOFORM CHANGES OCCUR WITHIN 1-4 HOURS
   - CARDIAC CATHETERIZATION
   - CARDI OVERSION
   - MYOCARDITIS

III. CENTRAL NERVOUS SYSTEM DISORDERS
   - CEREBROVASCULAR ACCIDENT
   - SEVERE HEAD TRAUMA
   - REYE'S SYNDROME

IV. MALIGNANCIES (CK-1 ELEVATES)
   - CANCER OF COLON, ESOPHAGUS, LUNG, PROSTATE
**CK: MEASUREMENT**

**SAMPLE**

- Serum is preferred specimen, heparinized plasma may be used but results are less reliable.

- Activity is lost in vitro as active site sulfhydryls are oxidized; activity can be restored by incubation with N-acetylcysteine.

- CK activity sensitive to light.

- Activity stable for 1 week if stored in the dark at 4 °C, and 1 month frozen (assuming reagent contains sulfhydryl).

- Hemolysis falsely increases CK measured levels due to high concentration of reaction intermediates in RBC; trace hemolysis tolerable.

**ROSALKI MODIFIED METHOD**

\[
\text{Creatine phosphate + ADP} \xrightarrow{\text{pH 6.7}} \text{creatine + ATP}
\]

\[
\text{ATP + glucose} \xrightarrow{\text{HK}} \text{glucose-6-phosphate + ADP}
\]

\[
\text{Glucose-6-phosphate + NADP} \xrightarrow{\text{G6PD}} 6\text{-phosphogluconate + NADPH + H}^+
\]

**METHOD INTERFERENCE**

2 ADP \xrightarrow{\text{AK}} ATP + AMP

Adenylate kinase can be partially inhibited by excess AMP and/or diadenosine pentaphosphate (DAPP).

Hemolysis releases adenylate kinase from RBC – exhausts the AK inhibitors.
CK: MEASUREMENT

ISOENZYME FRACTIONATION

• MANY TECHNIQUES FOR FRACTIONATING CK ISOENZYMES

• ELECTROPHORESIS IS THE REFERENCE METHOD BUT IS TIME CONSUMING AND REQUIRES EXPERIENCE TO INTERPRET SOME PATTERNS

• SANDWICH IMMUNOASSAY FOR CK-MB (CK-2) MAY BE MOST SENSITIVE FOR AMI AND CAN BE AUTOMATED

A, The electrophoretic separation of serum CK isoenzymes from a healthy adult (left) and an individual (right) who had a myocardial infarction 24 hours previously. B, A diagram representing the CK isoenzymes.
CK ISOFORM FRACTIONATION

PRE-MI
1 HR POST-MI
4 HR POST-MI
24 HR POST-MI

ISOENZYME FRACTIONATION

- SANDWICH IMMUNOASSAY MEASURES MASS CONCENTRATION OF CK-MB (CK-2)
- HEALTHY INDIVIDUALS HAVE < 5 μg/L CK-MB
- IF CK-MB > 5 μg/L, THEN MUST DETERMINE THE CK RELATIVE INDEX
- CK RELATIVE INDEX = (CK-MB (μg/L)) / (TOTAL CK ACTIVITY (U/L)) x 100
- CK RELATIVE INDEX: < 4% IN SKELETAL MUSCLE DAMAGE > 4% IN M.I.

CK: MEASUREMENT