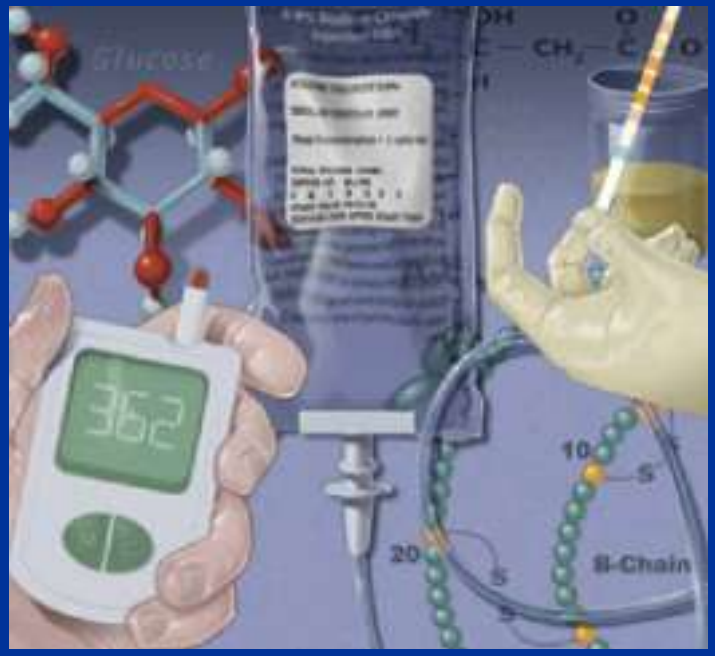
β-hydroxybutyrate: Past, Present and Future



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- Patrick O'Donnell D.O.
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- Resident in Pathology, on Chemistry Rotation
- Conducted literature search
- Drafted much of this presentation for a case presentation while on rotation

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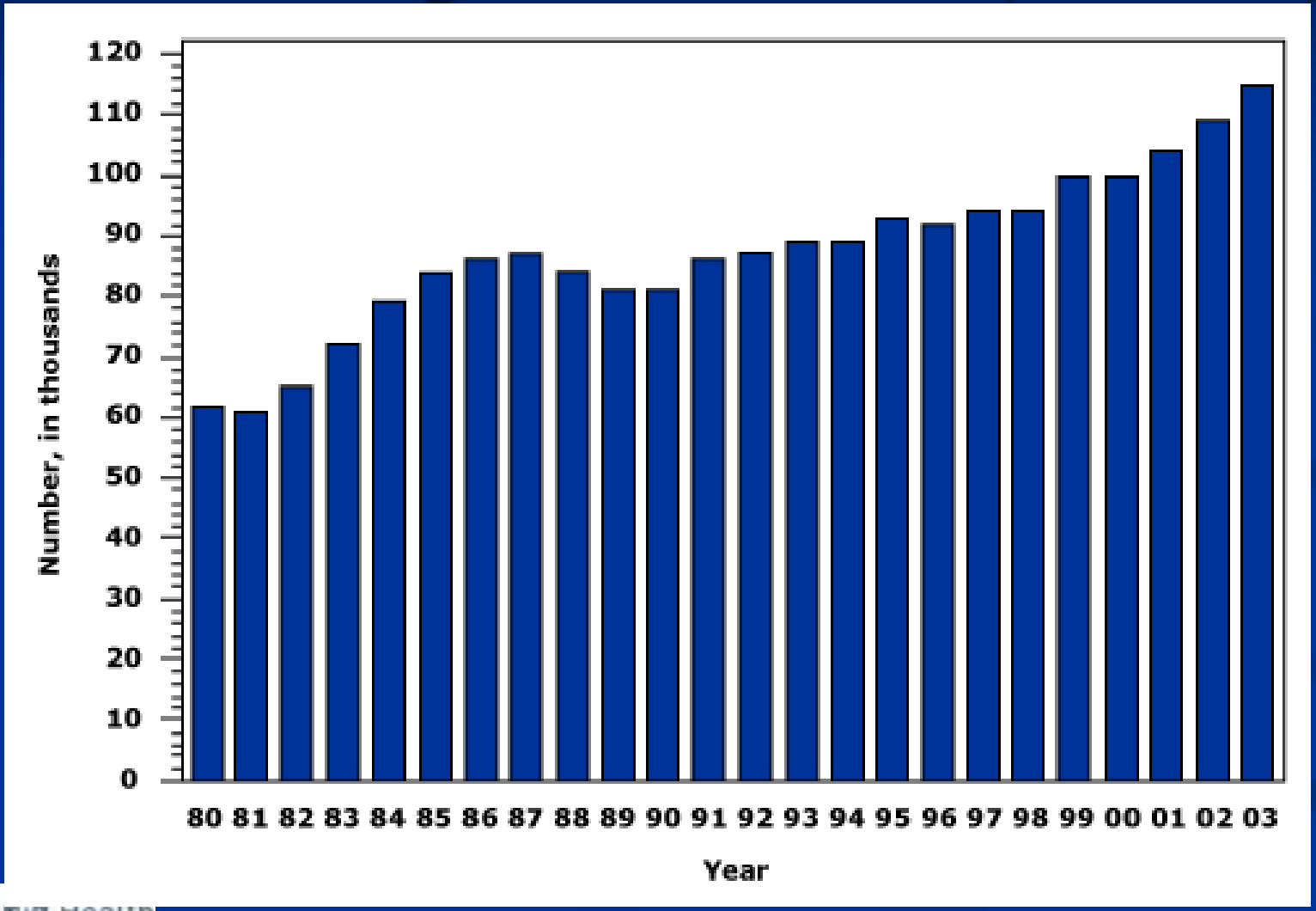
Diabetic Ketoacidosis

- Life-threatening complication of untreated diabetes mellitus (chronic high blood sugar)
- Insulin deficiency and stress hormones combine to cause DKA
- Was once the leading cause of death among Type I diabetics before insulin was available
- Characterized by hyperglycemia, acidosis and ketone bodies.

DKA Epidemiology

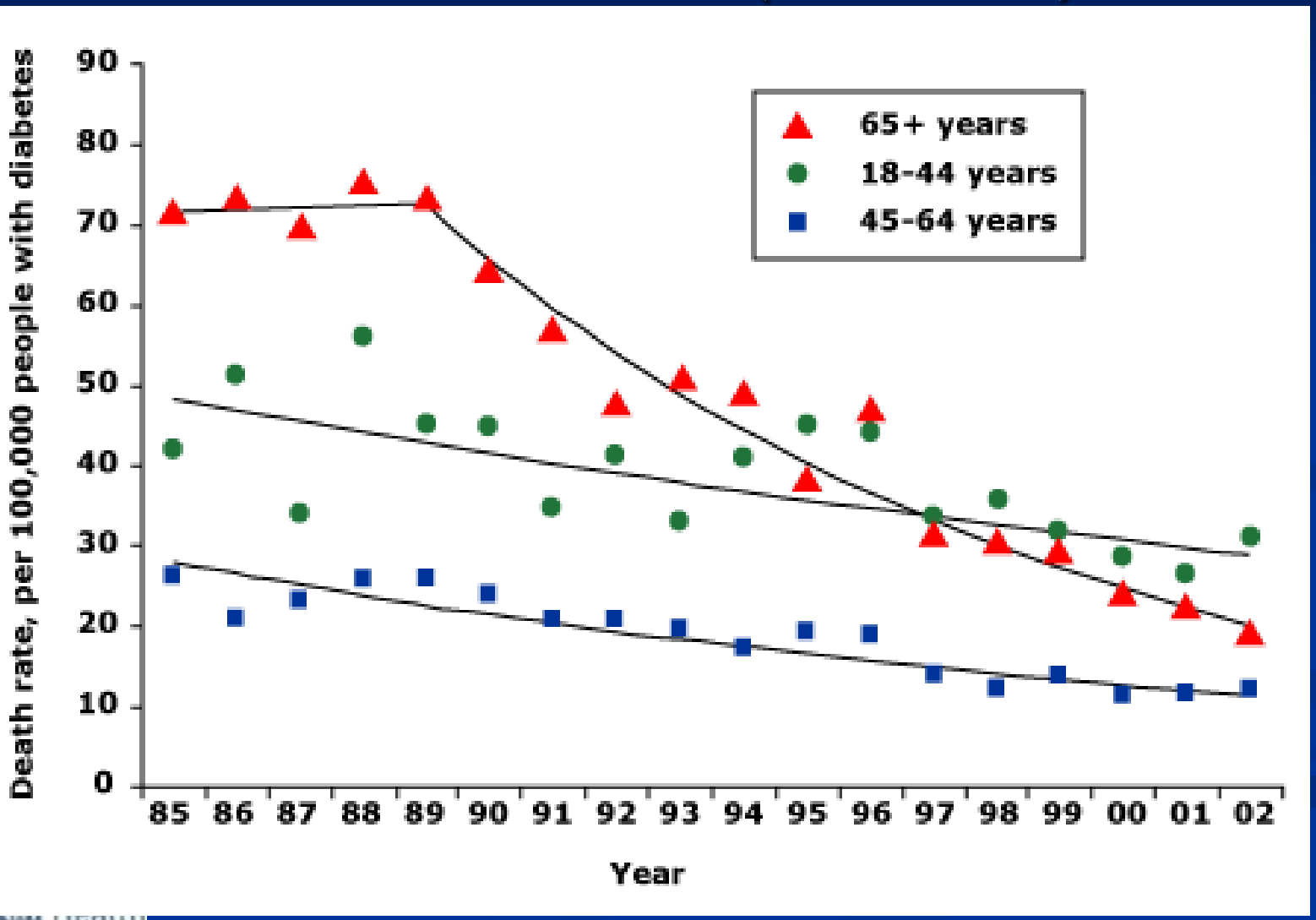
- **Type I Diabetes**
- Rarely Type II Diabetes in patients under extreme stress (serious infection, trauma)
- Young>Old, F>M (most common cause of death in diabetics under <20 y/o)
- $1 out of every $4 spent on direct medical care for adult patients with Type I DM
- Annual hospital costs in U.S. over $1 billion
- Mortality in DKA most commonly due to underlying precipitating illness and NOT due to metabolic consequences of hyperglycemia or ketoacidosis
- In 2003 CDC Nat'l DM Surveillance Program : 115K discharges for DKA in the U.S.

Number of hospital discharges with DKA as first listed diagnosis in the U.S. (1980-2003)



CDC, National Diabetes Surveillance System. 2005

Age Specific Death Rates for Hyperglycemic Crisis in the U.S. (1985-2002)



Wang J, et al. Diabetes Care 2006;29:2018.

Clinical Presentation

- Classic triad of polydipsia, polyuria, polyphagia
- Vomiting, abdominal pain
- Increased or deep respirations (Kussmaul)
- Signs of dehydration
- Weight loss, muscle wasting
- Fruity/medicine breath
- Cerebral edema
- CNS depression/coma

Typical Case

- 9 yo boy presents to clinic with " 6 day history of stomach pain and diarrhea." "Vomiting started 2 days ago and has persisted."
  - (+) weight loss
  - PE: HR 140, RR 28, T97.8 Weight: 27 Kg (59 lbs)
    - Tacky mucous membranes
    - Abd - soft, (+)BS, mild left tenderness
  - DX: viral gastroenteritis with mild dehydration
- Returned to ER 24 hours later
  - PE: cachectic (low weight), quiet, tired, uncooperative, (+) ketotic breath

Etiology

- DKA violates rules of common sense
  - Increased insulin requirement despite decreased food intake
  - Marked urine output in setting of dehydration
  - Catabolic state in setting of hyperglycemia and hyperlipidemia

Pathogenesis

- Two major causes of hyperglycemia and ketoacidosis in uncontrolled diabetics
  1. **Insulin deficiency is the primary defect**
  2. Glucagon excess
- Normal patients
  - Increased glucose >> **Insulin** release by pancreatic Beta cells reduces glycogenolysis and gluconeogenesis by the liver
    - Increase glucose uptake by skeletal muscle and adipose tissue
    - Insulin inhibits glucagon secretion directly and at the gene level in pancreatic alpha cells

Pathogenesis, cont.

- DKA is precipitated by stress
  - Increase the secretion of glucagon and cortisol and catecholamines
  - Some common "stressors":
    - Pneumonia, gastroenteritis, UTI, pancreatitis, MI, stroke, trauma, **alcohol and drug abuse**

Pathophysiology	Hormone
• Impaired insulin secretion	Epi
• Anti-insulin action	Epi, cortisol, GH
• Promoting catabolism	All
• Dec glucose utilization	Epi, cortisol, GH

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Andrew J. Bauer. Diabetic Ketoacidosis Gran Grounds. Walter Reed Army Medical Center. www.nccpeds.com/powerpoints/DKA.ppt#257,1,DIABETICKETOACIDOSIS

Pathogenesis, cont.

- Serum glucose of DKA usually <800 mg/dl
- Hyperglycemia in DKA due to 3 main processes:
  1. Impaired glucose utilization in peripheral tissues
  2. Increased glycogenolysis
  3. Increased gluconeogenesis

     -hepatic gluconeogenesis promoted by

     (1.) increased delivery of precursors (alanine, glycerol) due to fat and protein breakdown

     (2.) increased secretion of glucagon due to loss of inhibition by low insulin levels
- Glucosuria in DKA initially minimizes rise in serum glucose
  - Osmotic diuresis caused by glucosuria leads to volume depletion and decreased GFR that limits additional glucose excretion in the urine

Islets of Langerhans

β-cell destruction

Insulin Deficiency

Stress

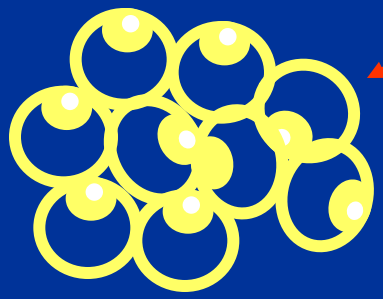
Epi, Cortisol
GH

Decreased Glucose Utilization &
Increased Production

Amino Acids

Increased Protein Catabolism

Glucagon



Increased Ketogenesis Gluconeogenesis, Glycogenolysis

FattyAcids

IncreasedLipolysis

Polyuria
Volume Depletion
Ketonuria

Threshold
180 mg/dl

Hyperglycemia
Ketoacidosis
HyperTG

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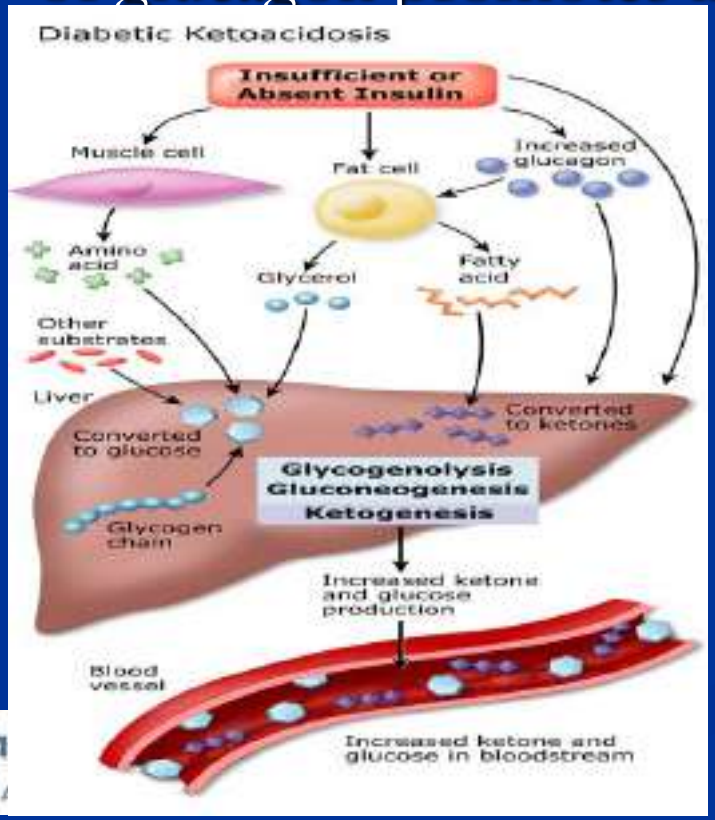
Pathogenesis, ketoacidosis

- Insulin deficiency causes increased lipolysis which increases FFA delivery to the liver
  - shuttled to the mitochondria, combined with effects of glucagon promotes ketone synthesis

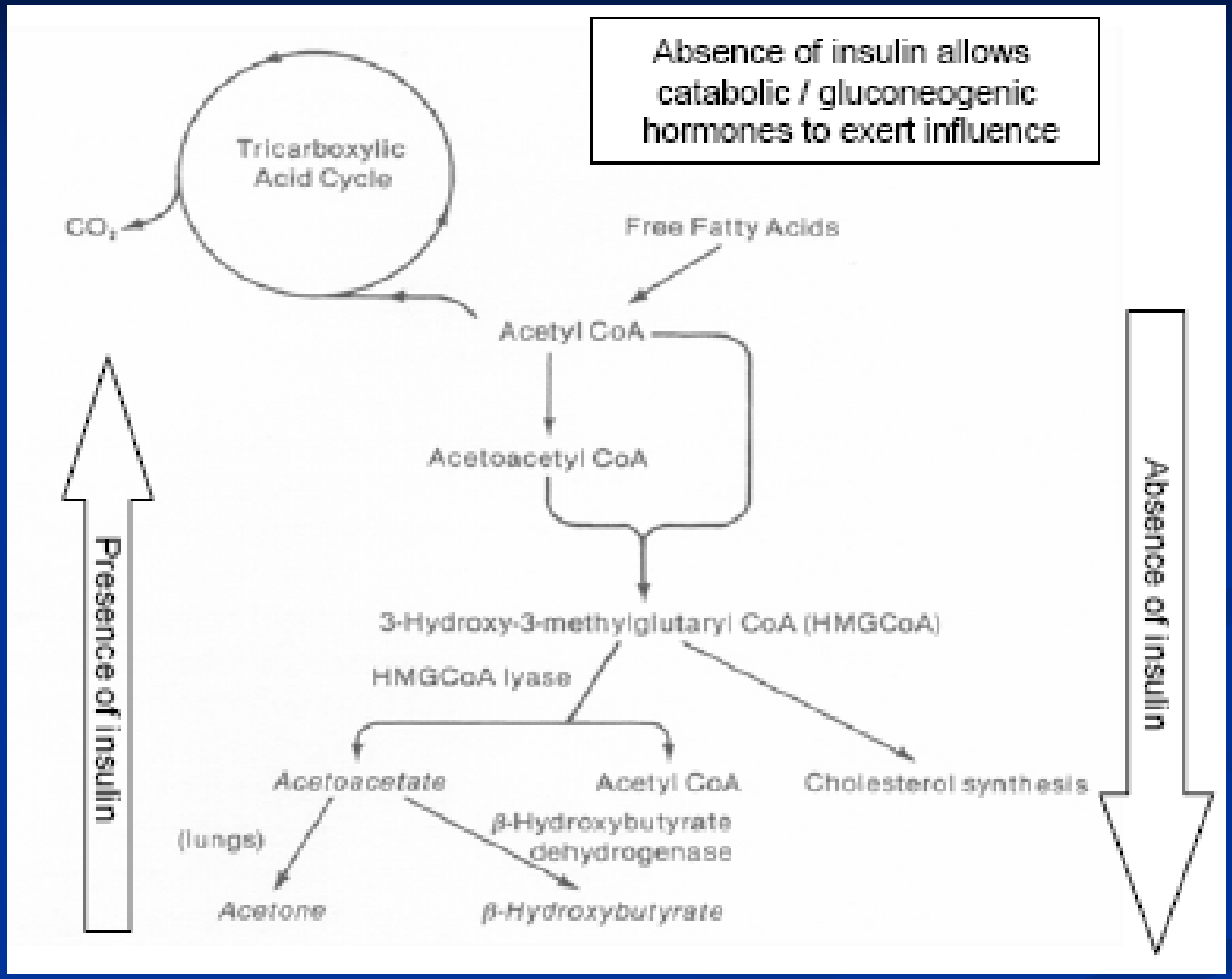


- Major ketones produced are **acetoacetic acid** and **β - hydroxybutyric acid** and **acetone**

- Normally a 1:1 of Acetoacetate:βOHB

Watermark Animation and Illustration.
dtc.ucsf.edu/images/illustrations/5.e_rev1.jpg

In DKA, the ratio of Acetoacetate:βOHB shifts to 1:6.



Larry Kaplan. Laboratory Challenges to Diabetic Care. www.columbia.edu/itc/hs/medical/selective/advclinicalPathology/2005/lecture/DiabeticCareKaplanBW.pdf

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Laboratory Evaluation

- Severity of DKA is determined primarily by the pH, bicarbonate, and mental status, not glucose

TABLE 2
Diagnostic Criteria for Diabetic Ketoacidosis and Hyperosmolar Hyperglycemic State

	Mild DKA	*Moderate DKA*	*Severe DKA*	*HHS*
Plasma glucose (mg per dL [mmol per L])	> 250 (13.9)	> 250	> 250	> 600 (33.3)
Arterial pH	7.25 to 7.30	7.00 to 7.24	< 7.00	> 7.30
Serum bicarbonate (mEq per L)	15 to 18	10 to < 15	< 10	> 15
Urine ketones	Positive	Positive	Positive	Small
Serum ketones	Positive	Positive	Positive	Small
Beta-hydroxybutyrate	High	High	High	Normal or elevated[20]
Effective serum osmolality (mOsm per kg)*	Variable	Variable	Variable	> 320
Anion gap†	> 10	> 12	> 12	Variable
Alteration in sensoria or mental obtundation	Alert	Alert/drowsy	Stupor/coma	Stupor/coma

DKA = diabetic ketoacidosis; HHS = hyperosmolar hyperglycemic state.

**—Effective serum osmolality = 2 × measured Na (mEq per L) + [glucose (mg per dL) ÷ 18].*

†—Anion gap = $Na^+ - (Cl^- + HCO_3^-$ (mEq per L)].

Adapted with permission from Kitabchi AE, Umpierrez GE, Murphy MB, Barrett EJ, Kreisberg RA, Malone JI, et al. Hyperglycemic crises in diabetes. Diabetes Care 2004;27(suppl 1):S95, with additional information from reference 20.

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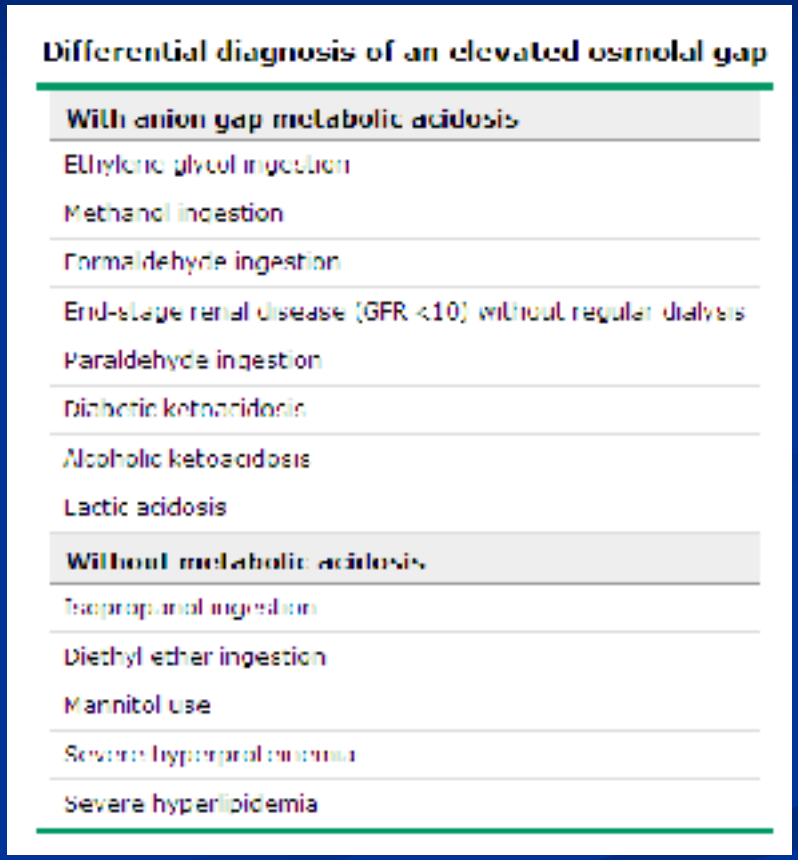
Trachtenburg DE. Diabetic Ketoacidosis. Am Fam Physician 2005;71:1705-22.

Laboratory Evaluation

- Serum Osmolality (mOsm/kg)
  - 2 x Na(meq/l) + plasma glucose (mg/dl)/18 + BUN/2.8
  - If serum osmolality < 320 mOsm/kg think of etiologies other than DKA
- Metabolic Acidosis
  - Due to Ketones
  - Anion Gap
  - Na – (Cl + HCO3)
  - pH Low



Differential diagnosis of an elevated osmolal gap

With anion gap metabolic acidosis
Ethylene glycol ingestion
Methanol ingestion
Formaldehyde ingestion
End-stage renal disease (GFR <10) without regular dialysis
Paraldehyde ingestion
Diabetic ketoacidosis
Alcoholic ketoacidosis
Lactic acidosis
Without metabolic acidosis
Isopropanol ingestion
Diethyl ether ingestion
Mannitol use
Severe hyperproteinemia
Severe hyperlipidemia

UpToDate. Osmolal Gap. Burton D. Rose, MD. 2007.

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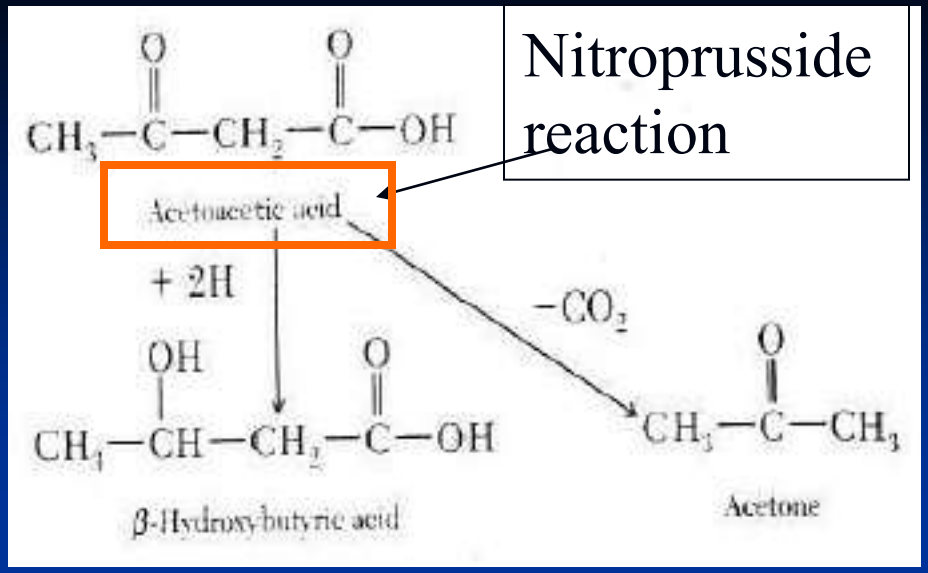
Electrolytes

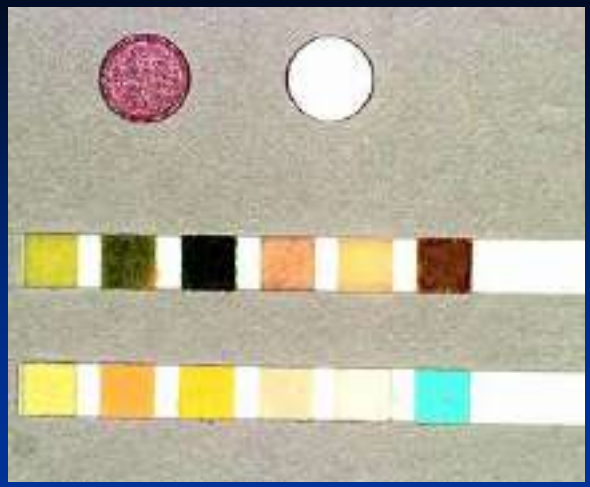
- Na
  - Depressed 1.6 mEq/l per 100mg% glucose increase
  - Depletion due to urinary losses/vomiting
  - Osmotic dilution
  - Remember hyperlipidemia can factitiously lower Na
- K
  - Serum K is often normal, but total body K is low
  - Can appear elevated due to lack of insulin and metabolic acidosis >> drives K extracellularly
  - SERIOUS issues can arise here with treatment…..K can bottom out!
- HCO_3
  - Always low in DKA
  - This extracellular ion is the body's first line buffer against metabolic acidosis

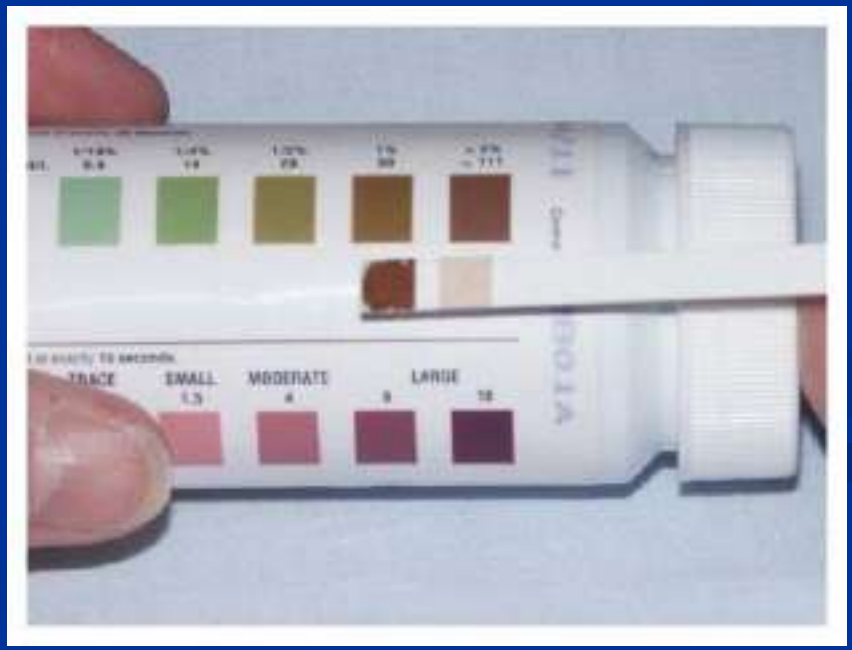
Ketone Bodies

- B-hydroxybutyrate accounts for >75% of the ketones seen in ketoacidosis
  - > 3mg/dl is abnormal
- Historically, ketoacidosis dx'd and monitored in urine and serum with nitroprusside based tests
  - Ketostix, Acetest (colorimetric visual interpretation-semiquantitative)
  - Nitroprusside based tests measure acetoacetate
  - Acetoacetate is not predominant ketone body in DKA

Nitroprusside reaction







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βOHB Quantitation

β-Hydroxybutyrate dehydrogenase

β-Hydroxybutyrate + nicotinamide adenine dinucleotide (NAD) ⟶ + acetoacetate + reduced NAD (NADH)

Diaphorase

NADH + nitroblue tetrazolium (oxidized, colorless) ⟶ NAD + nitroblue tetrazolium (reduced, colored)

Purple color (580nm) proportional to the concentration of βOHB

Normal: 0 – 0.3 mM/l

Ketosis: greater than 0.3mM/l

Possible ketoacidosis: greater than 5mM/l

Ketone Bodies, cont.

- In severe ketoacidosis:
  - βOHB:acetoacetate favors βOHB, nitroprusside test could be negative or weakly positive despite severe ketoacidosis
- When ketoacidosis improves the βOHB : acetoacetate favors acetoacetate, nitroprusside tests will have a stronger reaction even though ketoacidosis is actually improving
  - Fall of acetoacetate lags behind the improvement of ketoacidosis
- Drugs can cause a false positive nitroprusside test
  - ACEi

Ketone Bodies, cont.

- According to the American Diabetes Association - … "currently available urine ketone tests are not reliable for diagnosing or monitoring treatment of DKA"
- Testing for blood βOHB
  - Quantitative test…can use to diagnose/monitor ketoacidosis
    - Site experiences (Henry Ford Hospital) reported decreased TAT
    - No subjectivity in test, Number vs subjectivity of color change
    - Reduction in laboratory testing in patients with ketoacidosis (monitor BOHB and anion gap for trends)
    - COST savings
  - Shorter triage time, faster time to diagnosis

- Other causes of ketoacidosis….
  - Malnutrition…alcoholism..
  - Alcoholics
    - Decreased carbohydrate intake (reduced insulin sec.)
    - Increased glucagon secretion
    - Alcohol induces inhibition of gluconeogenesis and stimulates lipolysis >>>increased ketoacids
    - High anion gap metabolic acidosis, elevated osmolal gap
    - Hyperglycemia can occur but not usually as high as the levels seen in DKA
    - If glucose is not elevated and βOHB increased , ketoacidosis due to starvation/alcoholism
    - Up to 90% of ketones can be due to βOHB

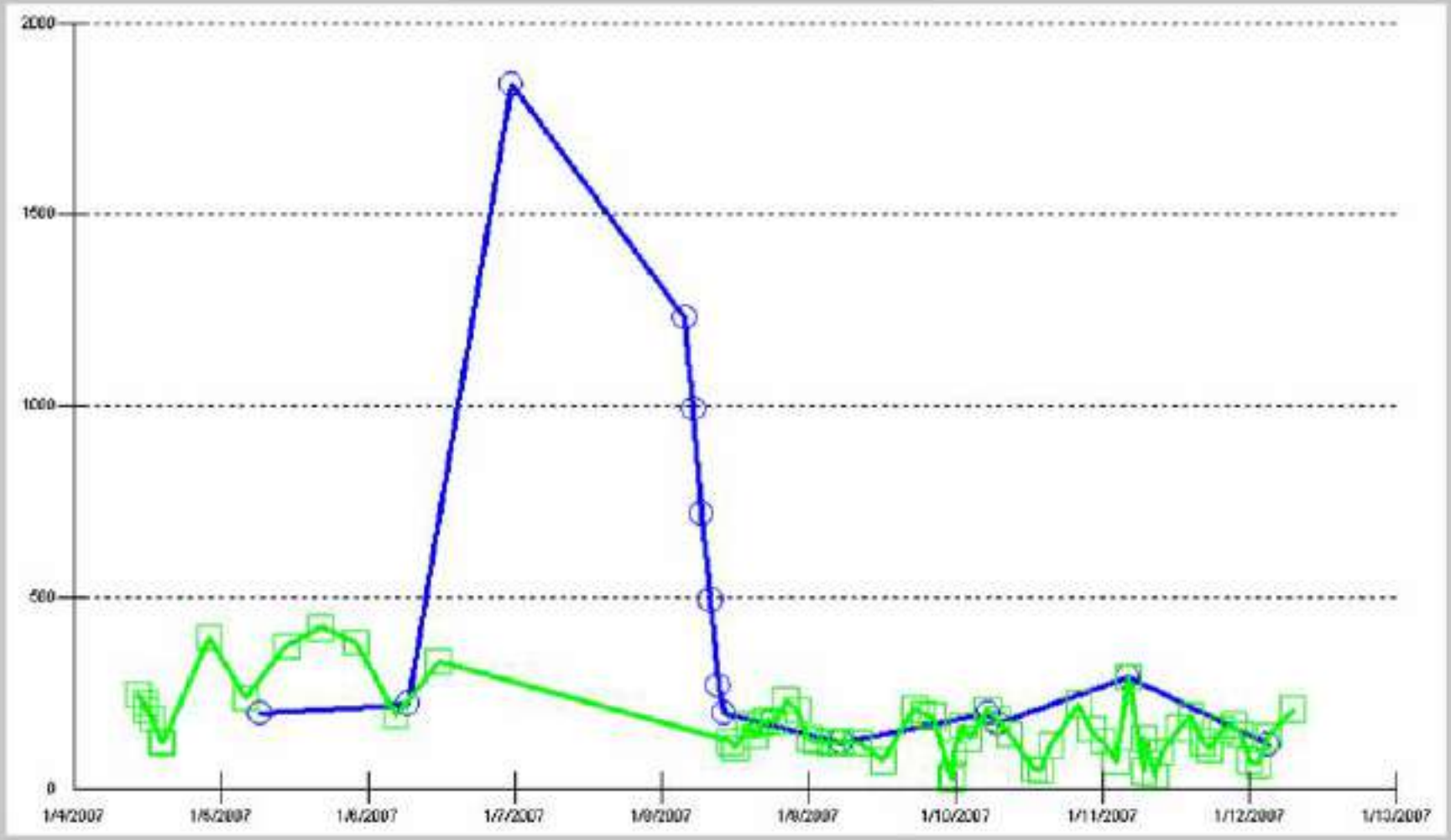
βOHB……other uses?

- Dx Pregnant patients
  - Dx gestational diabetes
- Monitoring DKA therapy
  - βOHB as an adjunct to monitoring diabetic control in addition to glucose testing

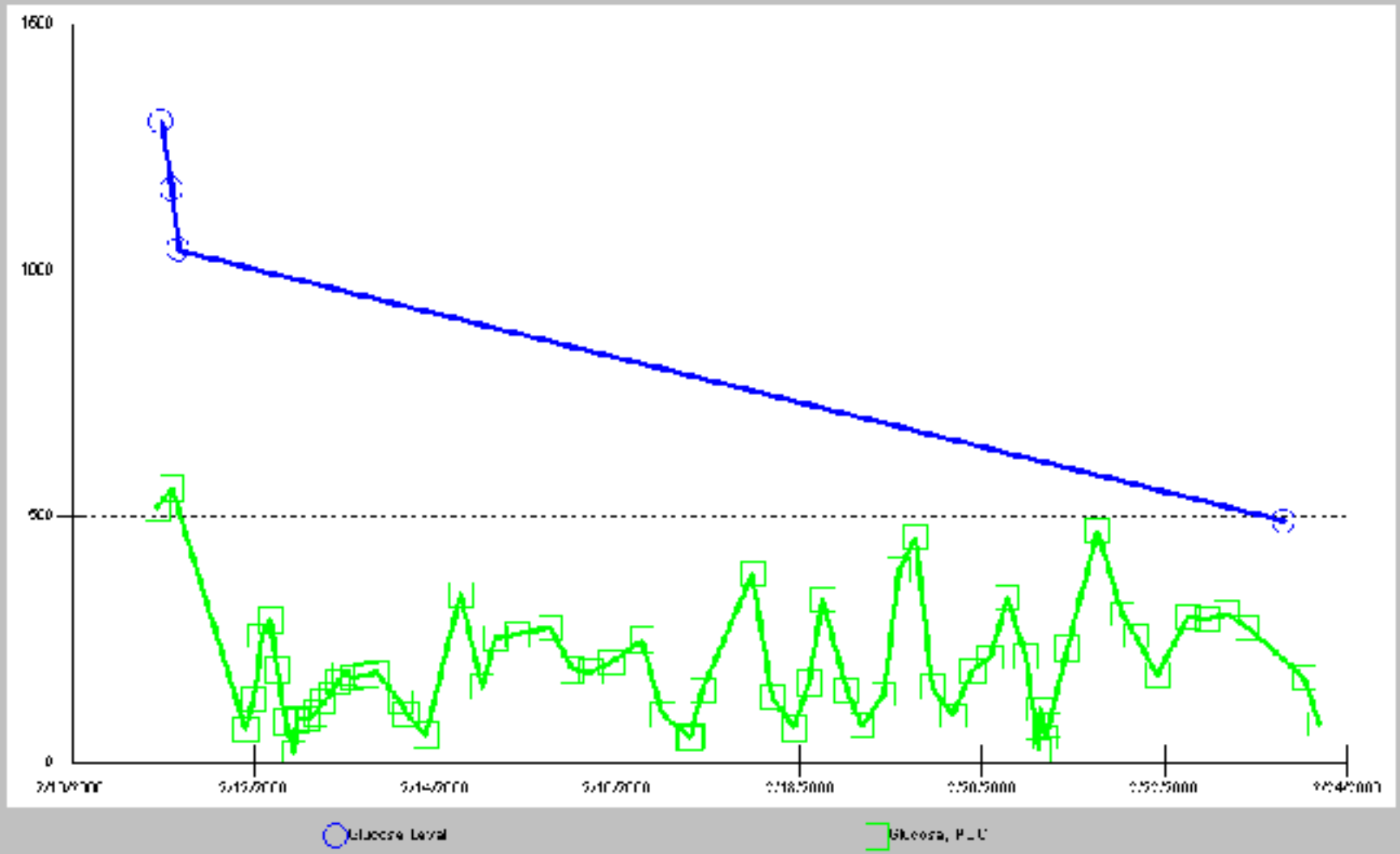
βOHB……other uses?

- Detect ketosis in ED!
  - Known limitation of glucose meters
  - Erroneous results reported for all current meters
  - Package insert example: "test results may be erroneously low if the patient is severely dehydrated or severely hypotensive, in shock or in a hyperglycemic-hyperosmolar state (with or without ketosis)
  - Cause unknown, several theories:
    - Poor peripheral circulation when in "shock"
    - Acidosis, ketone bodies or other interferent in circulation

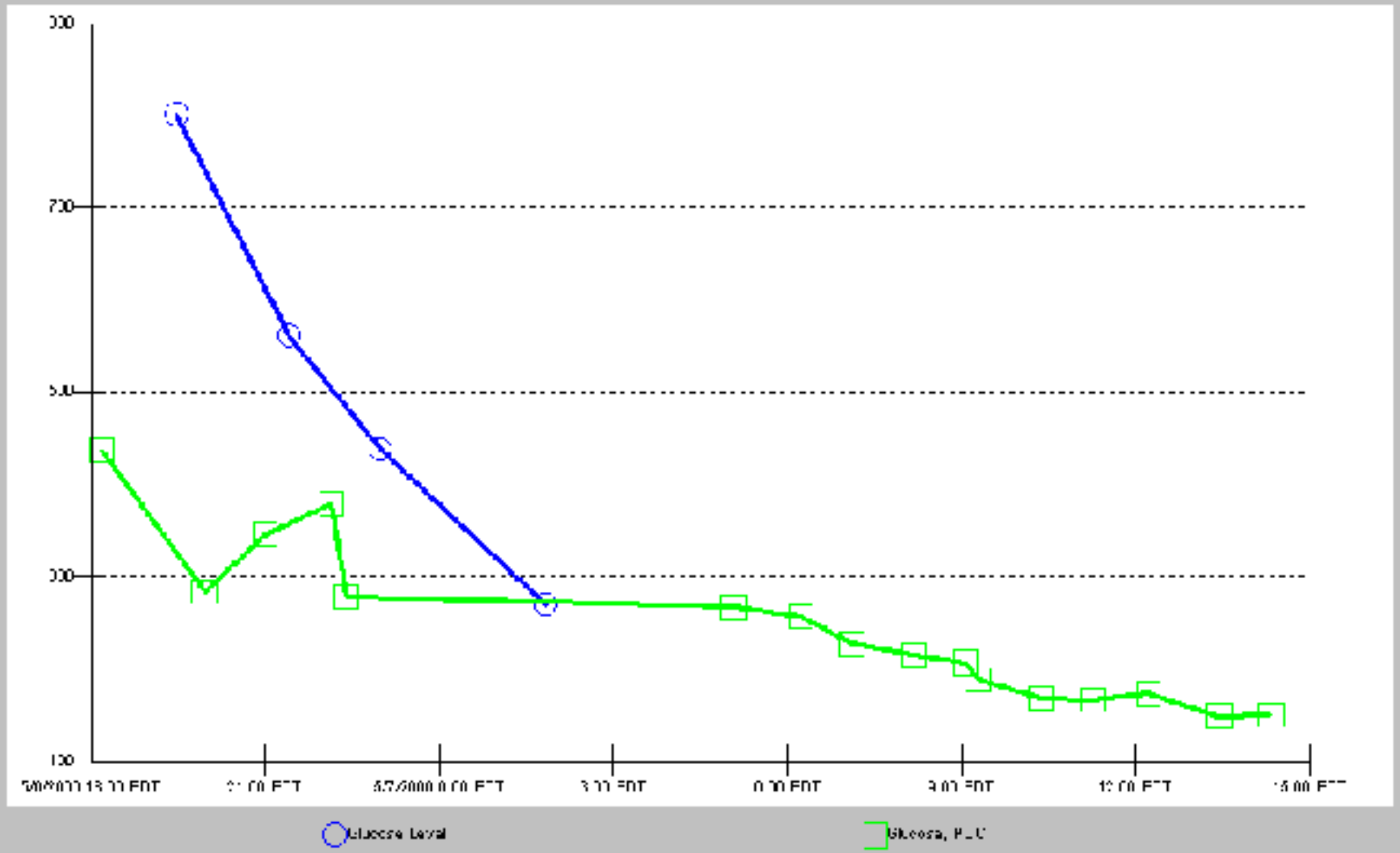
Glucose Level & Glucose, POC



Glucose Level & Glucose, POC



Glucose Level & Glucose, POC



DKA Glucose Meter Interference

- Baystate Medical Center ED study
  - 50 bed ED, Level 1 trauma and pediatric referral center
  - Over 100,000 visits annually
- ED staff need hourly glucose levels with rapid results to manage insulin dose of DKA patients
- Lab TAT approx 1 hour for stat testing, ED is drawing next sample without knowing results of previous sample
- Investigated differences of glucose meters vs lab results in DKA patients and whether an offset could be used to manage insulin.

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DKA Glucose Meter Interference

- Methods
  - 54 consecutive blood draws from suspected DKA patients
  - Green-top heparinized 3 mL blood sample
  - Drop of sample tested by glucose meter using Diff-Safe collection device without removing stopper
  - Send remainder to lab for stat analysis
  - Collect confirmed diagnosis, bicarb levels (degree of metabolic acidosis), and βOHB levels (ketonemia).

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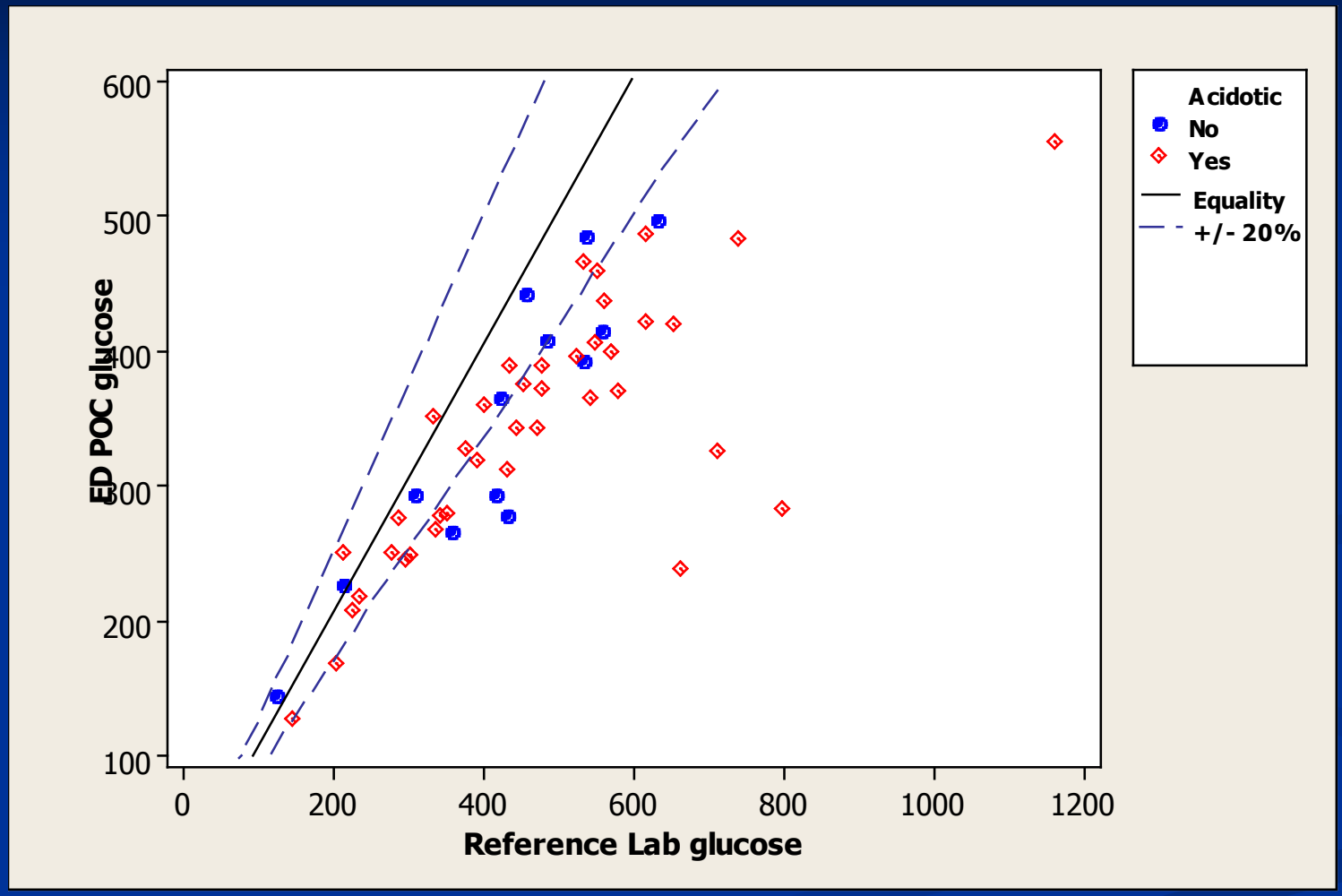
DKA Glucose Meter Interference

- Demographics
  - Age 10 – 86 years
  - 63% female
  - 46% final diagnosis of DKA
- Conclusions – "Use lab results when managing DKA pts"
  - POC glucose underestimated lab glucose in 50/54 cases (93%)
  - Only 52% of POC results within +/- 20% of lab value
  - Higher the glucose level, greater difference ($r=0.87$, $p<.0001$)
  - No association between acidosis and glucose correlation

POC vs Lab Glucose in DKA



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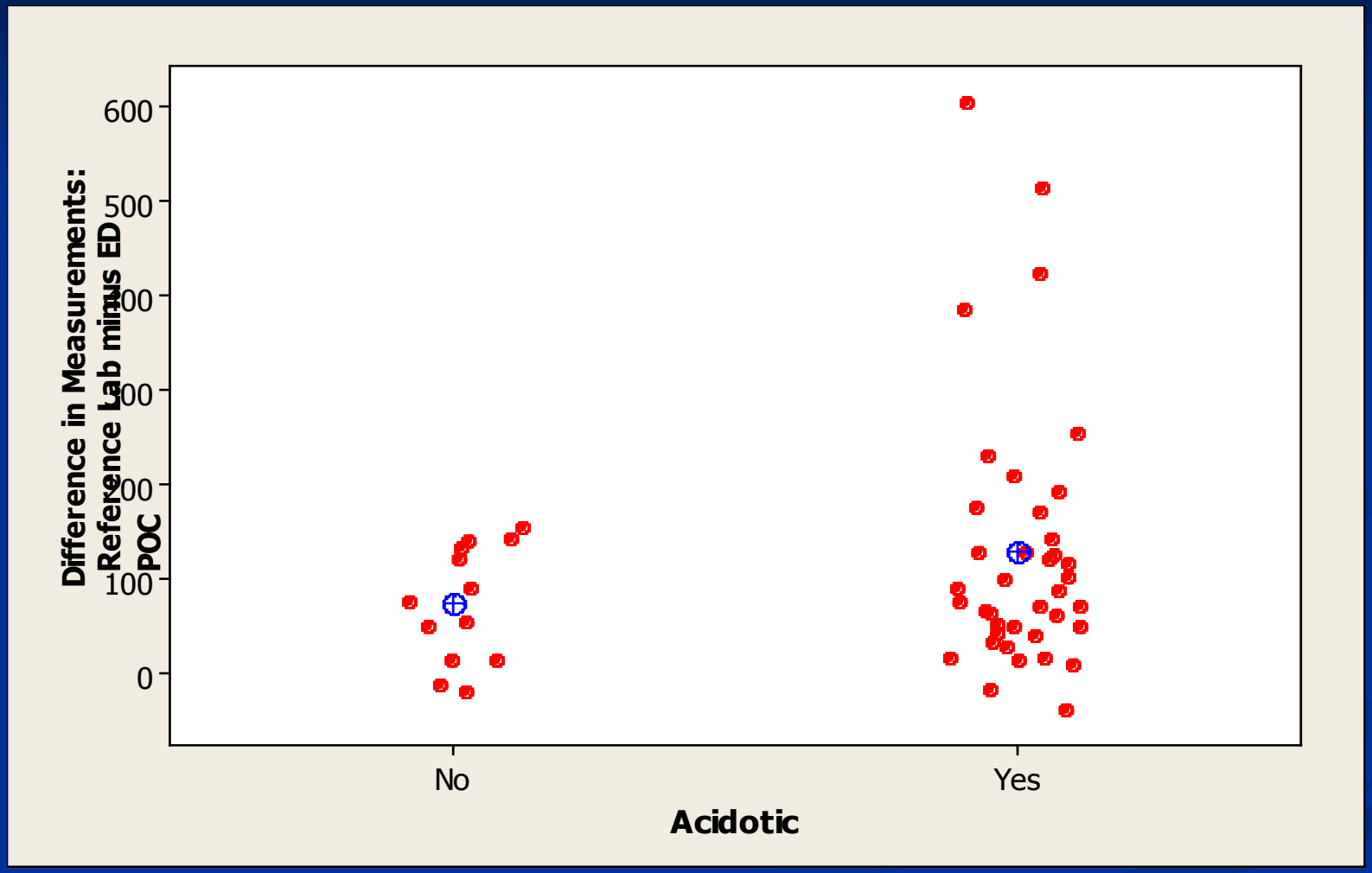
POC vs Lab Glucose in DKA



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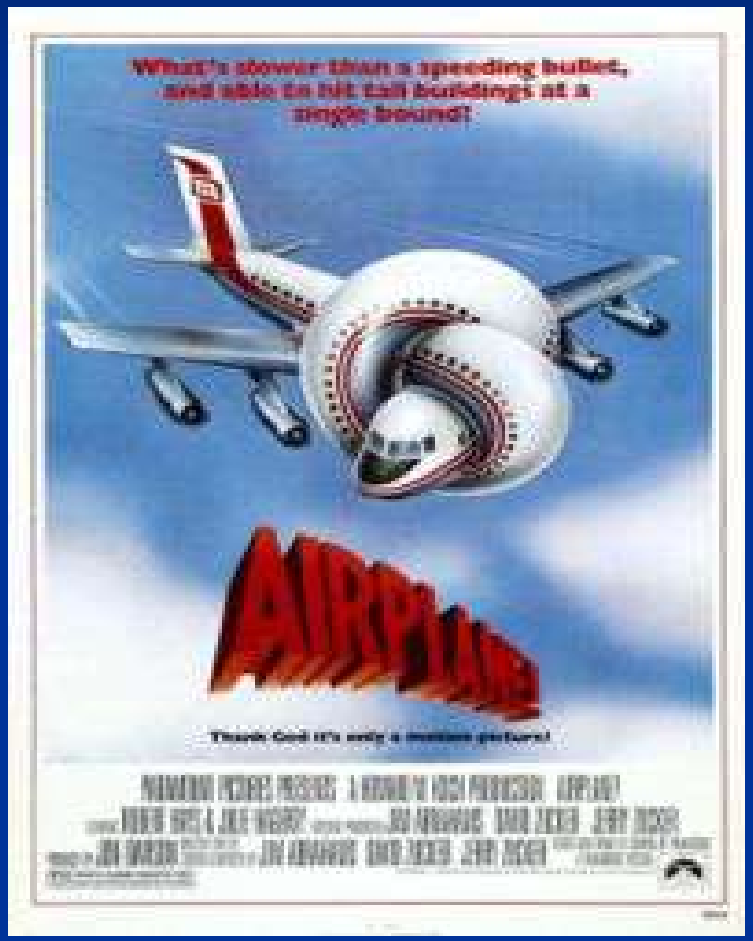
βOHB……other uses?



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Jim Abrahams

- The movies "Airplane" and "First Do No Harm" both share the same Producer/Director.
- Other Jim Abrahams movies include:
  - Big business (Bette Middler/Lily Tomlin)
  - Cocaine Blues (look at cocaine use starting with Sigmund Freud)
  - Coming to America (Eddie Murphy)
  - Cry Baby (A John Waters film with Johnny Depp)
- "First Do No Harm" was made for TV drama, outside Jim Abrahams typically movie genre.

"First Do No Harm"

- Ketogenic diet used since the 1920's….fell out of popularity with the development of anticonvulsants……
- Lennox-gastaeut syndrome – epilepsy refractive to drug therapy
- Meryl Streep (Lori) is met with narrow-minded resistance from Robbie's doctor, who is prepared to take legal action to prevent Lori from removing him from the hospital
- This movie is an indictment of those in the medical profession who discuss only the treatment options they favor
- The Charlie Foundation funded a multi-centre study that was published in 1996, which marked the beginning of renewed scientific interest in the diet.

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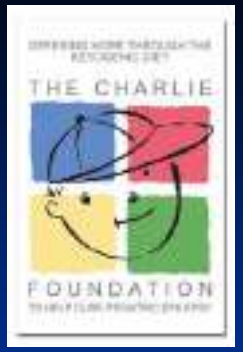
Not an FDA approved use of this test, off-label use of product.



The ketogenic diet and seizure control.

- High fat, adequate protein, low carbohydrate diet designed to mimic the effects of fasting.
- Increased ketone bodies….become the primary energy source for the brain.
- Well documented in unblinded studies to improve seizure control in children with difficult to control seizures
  - In general at least a 50% reduction in seizure frequency in 50% of the patients studied
  - Mechanism is unknown
- In the past monitored treatment compliance with nitroprusside based urine dipstick

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Not an FDA approved use of this test, off-label use of product.

Original Article

The Ketogenic Diet: Seizure Control Correlates Better With Serum β-Hydroxybutyrate Than With Urine Ketones

Donald L. Gilbert, MD; Paula L. Pyzik, BA; John M. Freeman, MD

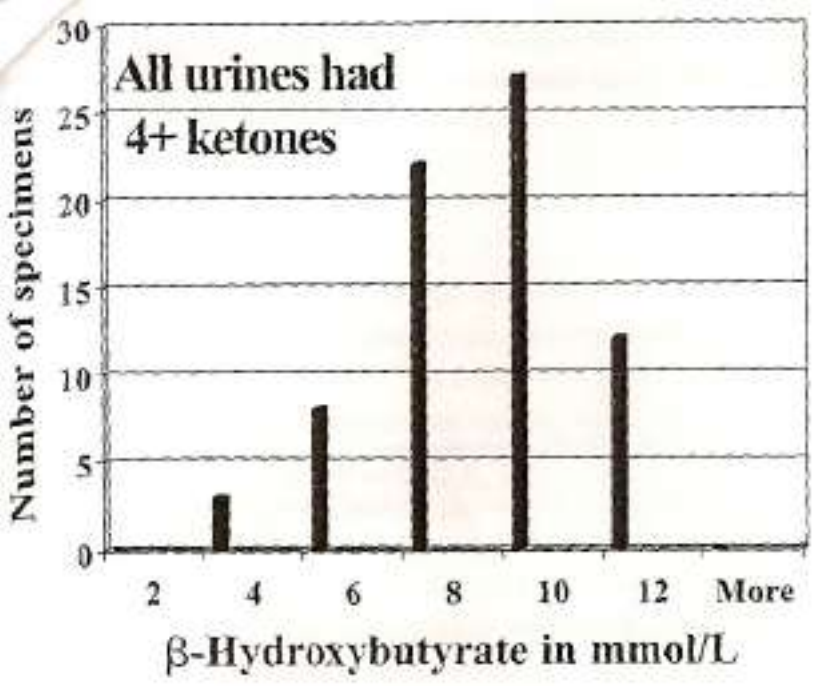

Table 2. Probability of Seizure Control 3 to 6 Months After Diet Onset (N = 54)

β-Hydroxybutyrate Level	*> 50% Control*	*> 90% Control*	*100% Control*
< 4 mmol/L (n = 12)	33%	8%	8%
> 4 mmol/L (n = 42)	83% (.002)*	48% (.018)*	43% (.039)*
< 5 mmol/L (n = 26)	62%	31%	27%
> 5 mmol/L (n = 28)	82% (.091)†	46% (.238)†	43% (.221)†
Any level	72%	39%	35%

*Above versus below the threshold of 4 mmol/L, children were statistically significantly more likely to achieve higher levels of seizure control (Fisher's exact *P* values, one cell with expected count less than 5).

†Above versus below the threshold of 5 mmol/L, children were not statistically significantly more likely to achieve higher levels of seizure control (chi square *P* values).

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Gilbert DL, et al. J Child Neurol. 2000;15:787-90.

Summary

- Past: Urine dipstick nitroprusside has historically been utilized to screen and monitor diabetic ketoacidosis – still widely marketed method.
- Present: Serum βOHB levels give a direct measurement of blood ketones, and are clearly a better method of Dx and managing ketosis
- Future: Potential uses of βOHB for managing compliance and personalizing antiepileptic and research into diets that promote ketosis.