UNIVERSITY OF AGRICULTURE
IN KRAKOW

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Why Do Cows Become Ketotic?

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Presentation Outline

What is Ketosis – defining the disease

Ketosis Pathogenesis – origin of ketones

Are ketone bodies detrimental?

Impact of ketones on the cow

Role of inflammation in ketosis

What is Ketosis?

- A metabolic state where ketone bodies can be detected
  - Does this suggest a disease state?
  - Is ketosis just associated with production of ketone bodies?
- A metabolic disease state where ketone bodies are detected in high concentrations and animal performance or health is compromised
  - Suggests a negative association with high ketone concentration
  - Does high ketones cause the disease?
- Disease is defined as a deviation from normal in structure or function of a living organism

What is Ketosis?

- Review by D.S. Kronfeld (JDS 1982) suggested three propositions:
  - Major determinant of milk yield is mammary uptake of glucose (1 kg milk = 72 g glucose)
  - Major determinant of lactational efficiency is mammary uptake of long-chain fatty acids
  - Spontaneous ketosis development is determined by ratio of glucogenic to lipogenic nutrients in diet
- Ketosis is accumulation of ketone bodies representing incomplete combustion of fatty acids during periods of lipid mobilization, which may or may not be dependent upon carbohydrate availability

What is Ketosis?

- These ketotic states are different from “spontaneous” ketosis
- Dietary (alimentary) ketosis
  - Consumption of highly ketogenic feed – ensiled forages
  - Clostridial fermentation of ensiled feeds, with high butyric acid content
- Underfeeding ketosis
  - Primary – limited feed availability inducing ketotic state; easily corrected by refeeding
  - Secondary – voluntary intake is diminished by disease inducing a ketotic state (e.g., hypocalcemia); addressing underlying disease can correct ketotic state

Kronfeld DS. J Dairy Sci 1982

Does elevated ketone bodies cause these clinical signs or reflect an underlying issue?

Clinical Ketosis

McArt and Nydam, Large Animal Internal Medicine, 6th ed, 2020

- Clinical signs – non-specific
  - Depressed appetite
  - Marked reduction in milk yield
  - Rapid body condition and weight loss
  - Decreased rumination, fill
  - Neurologic signs? – Pica, aggression
- Blood analytes
  - BHB > 2.9 mmol/L
  - Elevated NEFA
  - ± Hypoglycemia
  - Inflammatory mediators???

Are we Confusing Hyperketonemia and Ketosis?

- Terminology
- Hyperketonemia is elevated ketone concentrations above physiologic normal range
  - Hyper-keton-emia
  - Elevated ketone blood concentration
- Ketosis is recognized as a disease condition:
  - Ket: refers to ketone bodies
  - -osis: abnormal condition

- *What is normal range?*
- Diagnosis of ketosis has been reduced to defining a BHB threshold:
  - Clinical ketosis: [BHB] > 2.6 to 3.0 mmol/L
  - Subclinical ketosis: [BHB] > 1.2 to 1.4 mmol/L
- Disease should show clinical signs

Risk Factors for Ketosis

- Increasing parity
  - Older cows produce more milk
- Higher body condition score
  - Greater adipose reserve, ↓ DMI
- Season of calving
  - Spring, Summer greater risk
- Dry period length
  - Longer dry periods are at risk
- Previous lactation length

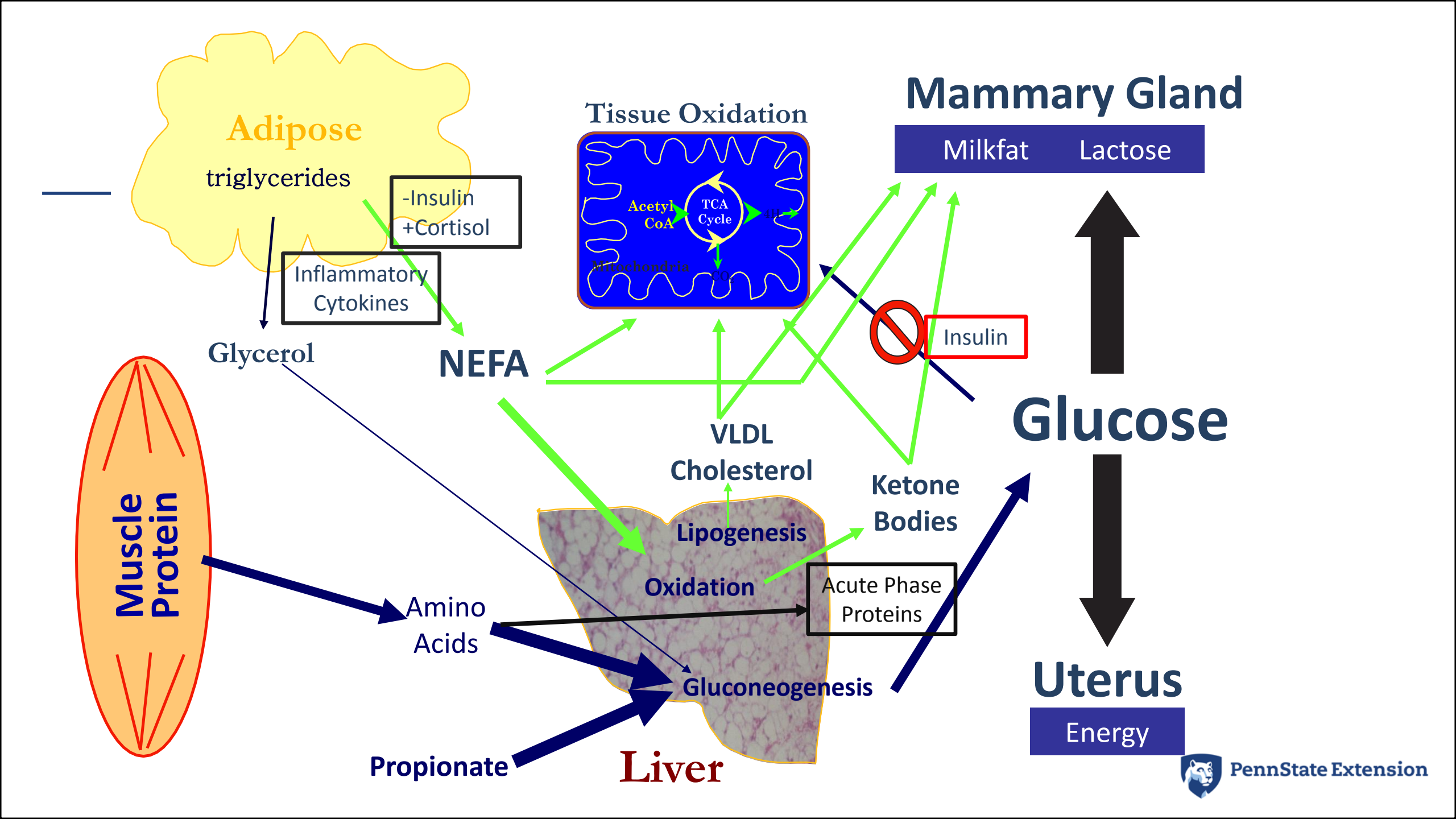
Vanholder et al., J Dairy Sci 98 (2015); McArt et al., J Dairy Sci 96 (2013); Pralle et al., Animals 11 (2011); Ha et. al. Vet Med Sci 1-8 (2022); Lean et. al., Res Vet Sci 57 (1994)

Ketosis Pathogenesis

- Cow experiences a state of negative energy balance
  - Decreased DMI
  - Increased energy nee
- Mobilization of adi
- Liver proces
  ketone bod
  - Loss of re esis?
  - Inability to EFA or export?
  - Metabolic dy gulation? ↓ OAA or altered redox status?

- thogenesis of y understood *2017*)
- ketones are not only ites affected in ketosis
- evated ketones (BHB) cannot account for clinical signs. What about acetone?
- Genetic factors? h^2 = 0.01-0.16
- Genes for cytokine signaling, fatty acid uptake/transport and fatty acid oxidation upregulated (*Loor et. al., 2007*)
- Role of inflammation???

"Current diagnosis method relying on ketone bodies is facing a crisis." Zhang and Ametaj, 2017


Adipose
triglycerides
-Insulin
+Cortisol
Inflammatory
Cytokines
Glycerol
NEFA
Tissue Oxidation
Acetyl
CoA
TCA
Cycle
4H
Mitochondria
CO
Mammary Gland
Milkfat
Lactose
Insulin
Glucose
VLDL
Cholesterol
Ketone
Bodies
Lipogenesis
Oxidation
Acute Phase
Proteins
Muscle
Protein
Amino
Acids
Gluconeogenesis
Propionate
Liver
Uterus
Energy
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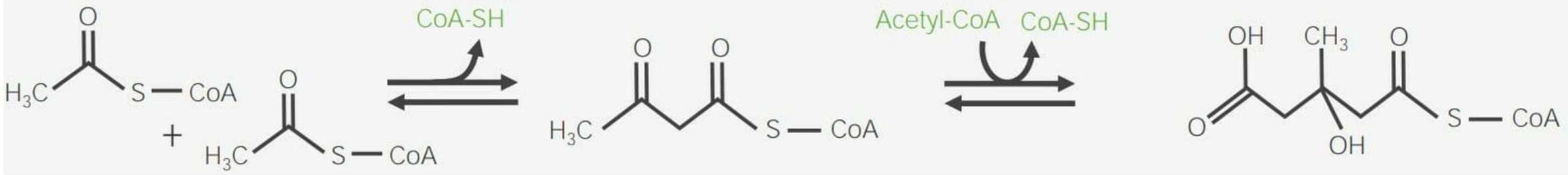
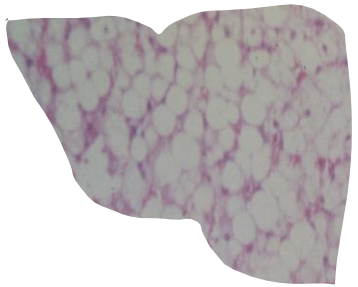
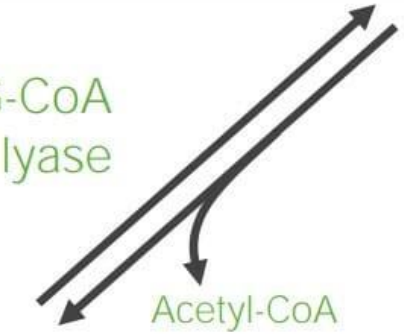
2 Acetyl-CoA — Thiolase → Acetoacetyl-CoA — HMG-CoA synthase → β-hydroxy-β-methylglutaryl CoA (HMG-CoA)



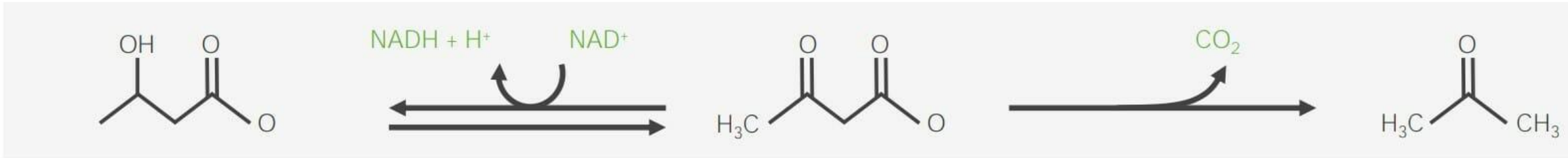


HMG-CoA lyase



β-hydroxybutarate dehydrogenase

Non-enzymatic decarboxylation



D-β-hydroxybutyrate — Reverse reaction — Acetoacetate — Spontanous, non-reverse reaction — Acetone

https://www.lecturio.com/concepts/ketone-body-metabolism/

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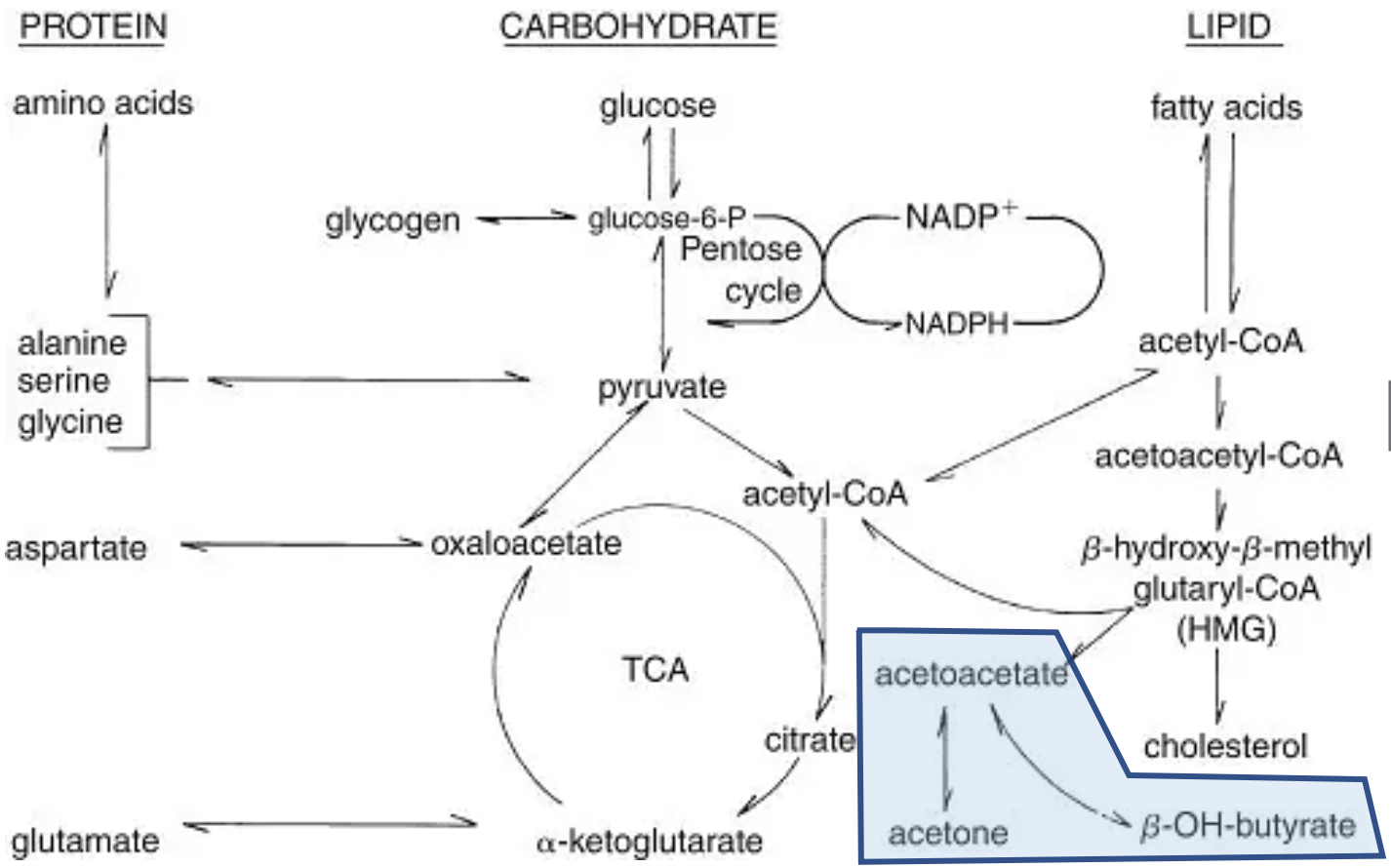
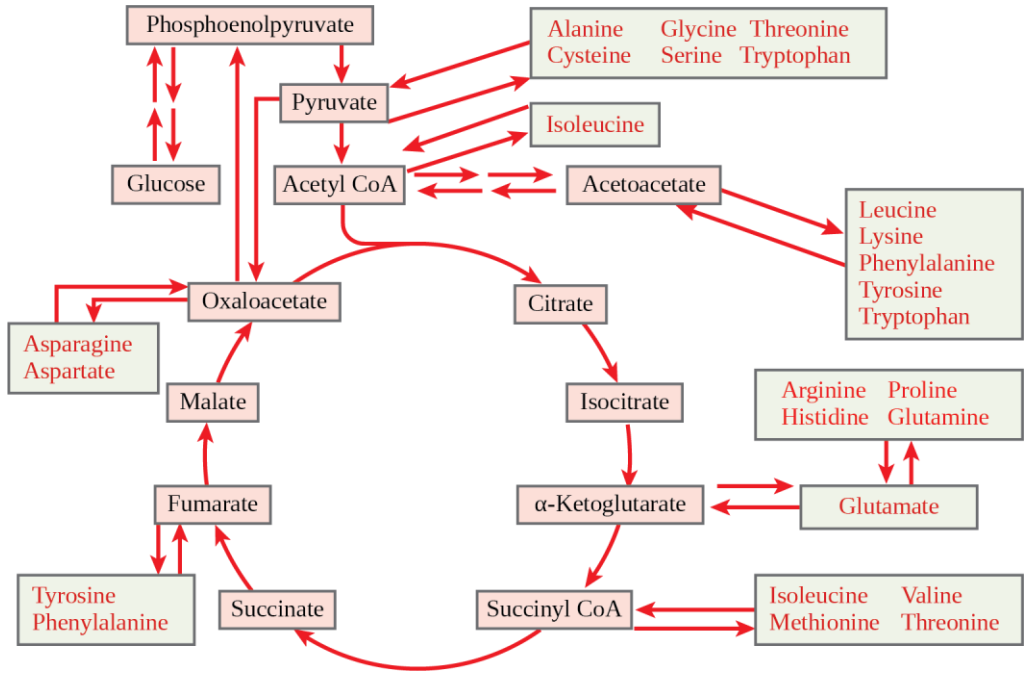
Integrated Metabolism





https://courses.lumenlearning.com/suny-osbiology2e/chapter/connections-of-carbohydrate-protein-and-lipid-metabolic-pathways/

https://veteriankey.com/carbohydrate-metabolism-and-its-diseases/

Metabolic Enzyme Adaptations

	Nonketotic Cows	Ketotic Cows
BHB, mmol/L	0.48 ± 0.22	1.98 ± 0.90
NEFA, mmol/L	272 ± 62	763 ± 152
Glucose, mmol/L	4.36 ± 0.85	2.05 ± 0.29

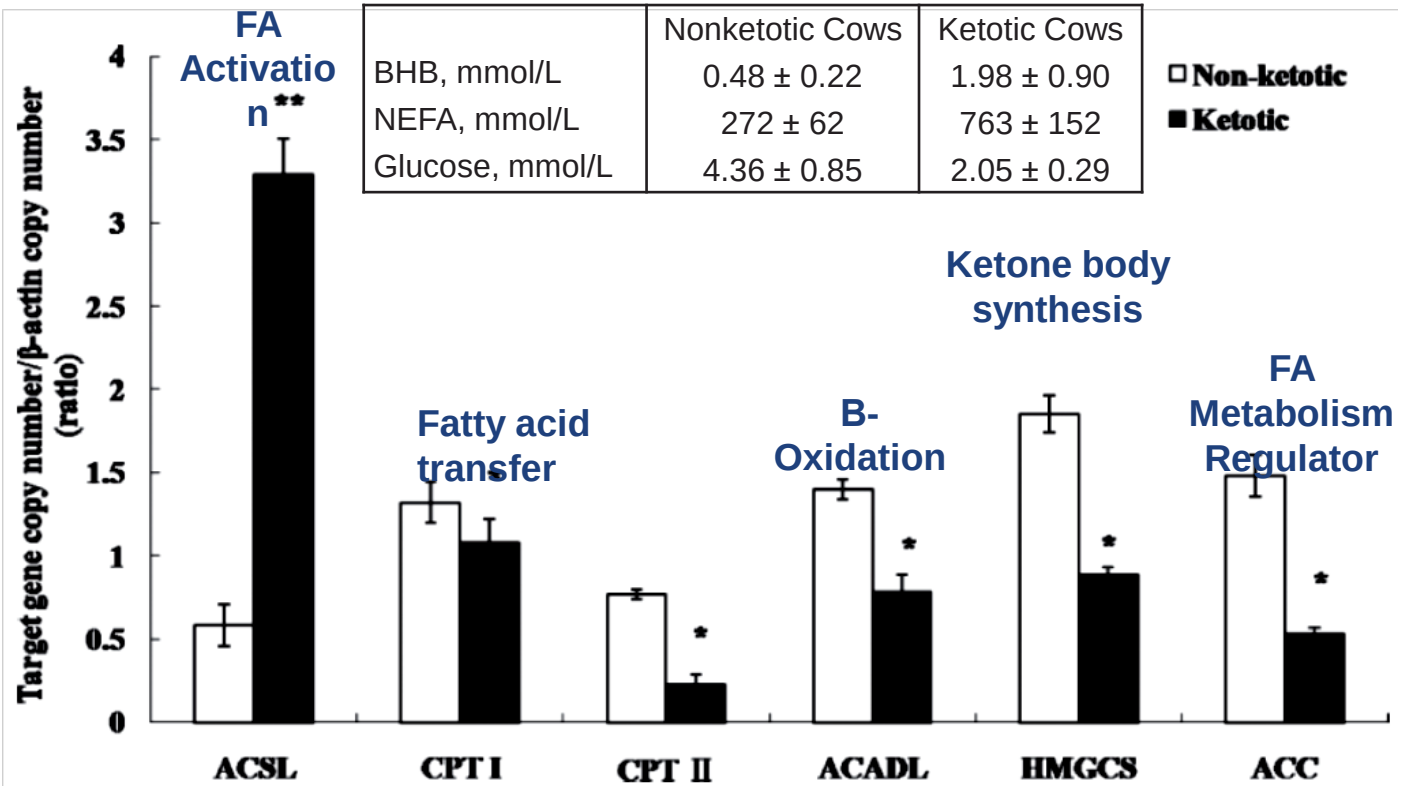


Figure 1. Real-time reverse transcription-PCR analysis of fatty acid metabolism-related enzymes in the liver. Values are reported as target gene mRNA copy number of 1 μg of total RNA/β-actin mRNA copy number. *$P < 0.05$; **$P < 0.01$.

- Evaluation of fatty acid metabolism associated enzymes
- Greater activation of fatty acids in ketotic cows
- Reduced capacity for oxidation of fatty acids
- Increases potential for triglyceride synthesis to induce fatty liver

Li et al., J Dairy Sci 95 (2012)

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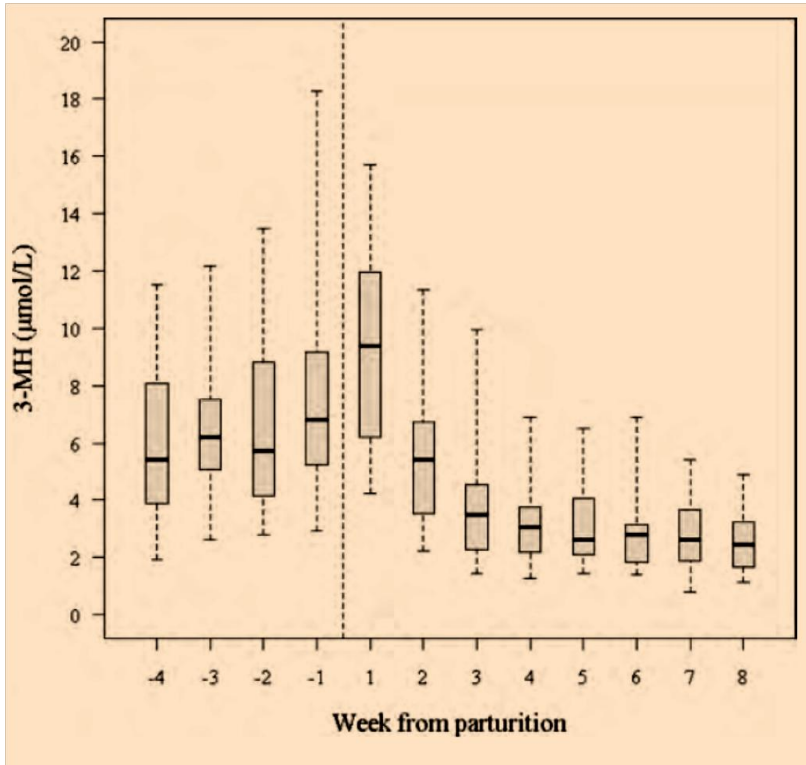
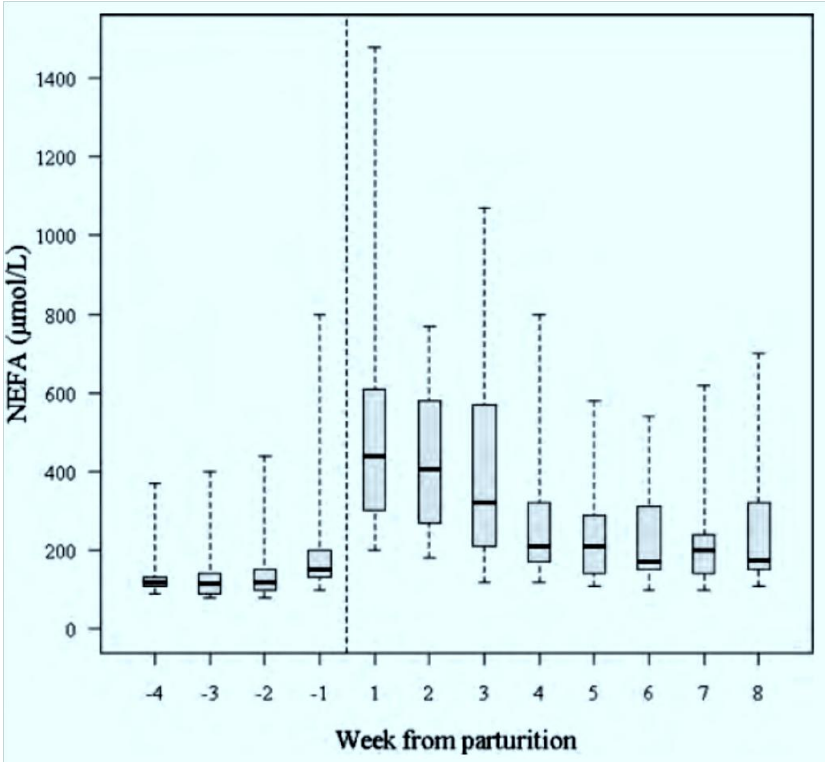
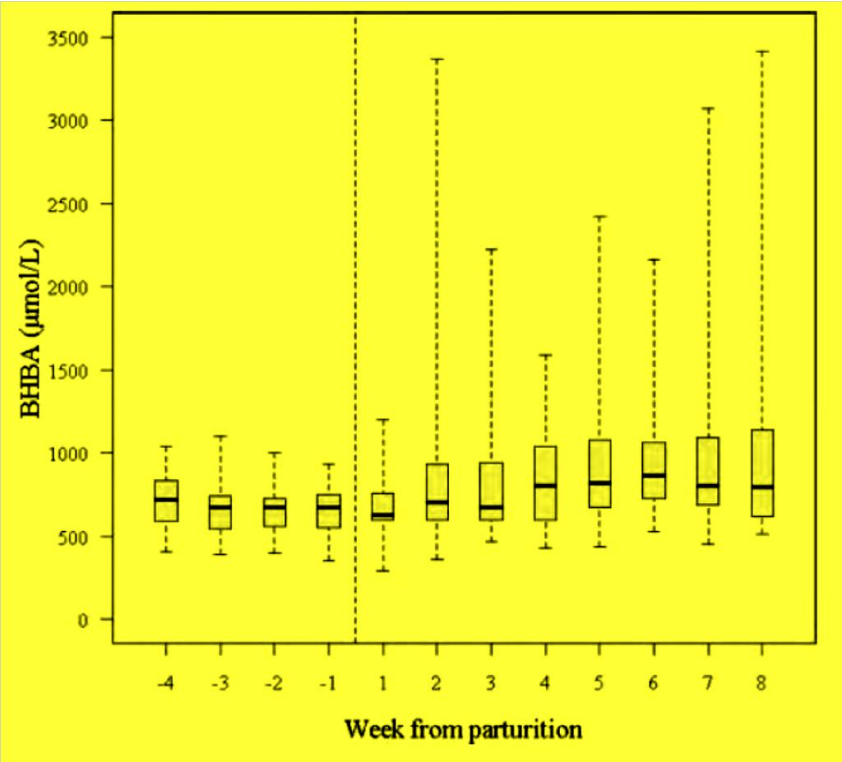
Origin of Ketones







- Lower plasma 3-methyl histidine (3-MH) concentrations were associated with a greater hyperketonemia
- Serum NEFA concentrations were not associated with degree of hyperketonemia
- Serum NEFA concentration were associated in cows with severe hyperketonemia
- Cows able to mobilize body protein had lower BHB concentrations

van der Drift et al., J Dairy Sci 95 (2012)

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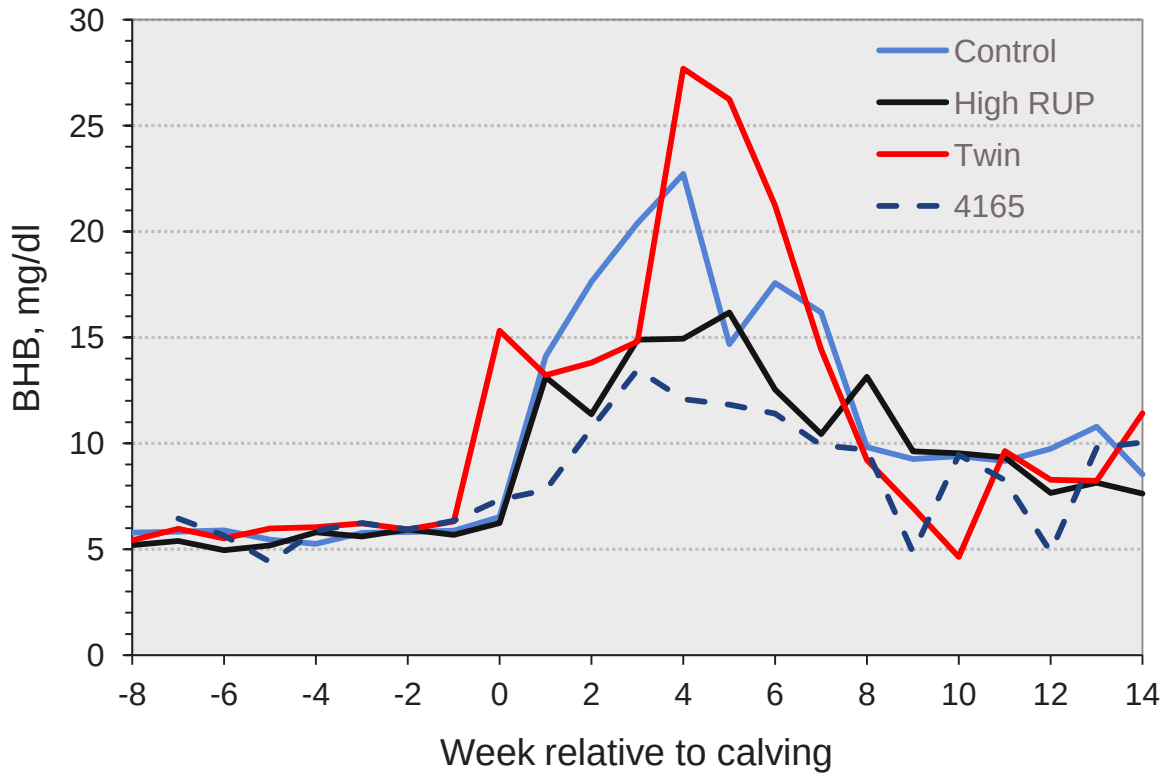
Effect of Protein on Ketogenesis



- Prepartum diets differed in amount of protein provided by RUP sources
- Control group cows experienced 31% clinical ketosis
- High RUP group did not present with clinical ketosis
- Cows with twins had clinical ketosis and other concurrent diseases
- Cow 4165 had twins, but fed the High RUP diet prepartum
- Postpartum diets were isocaloric and isonitrogenous

Van Saun, PhD Thesis 1993

Is Ketosis a Disease?

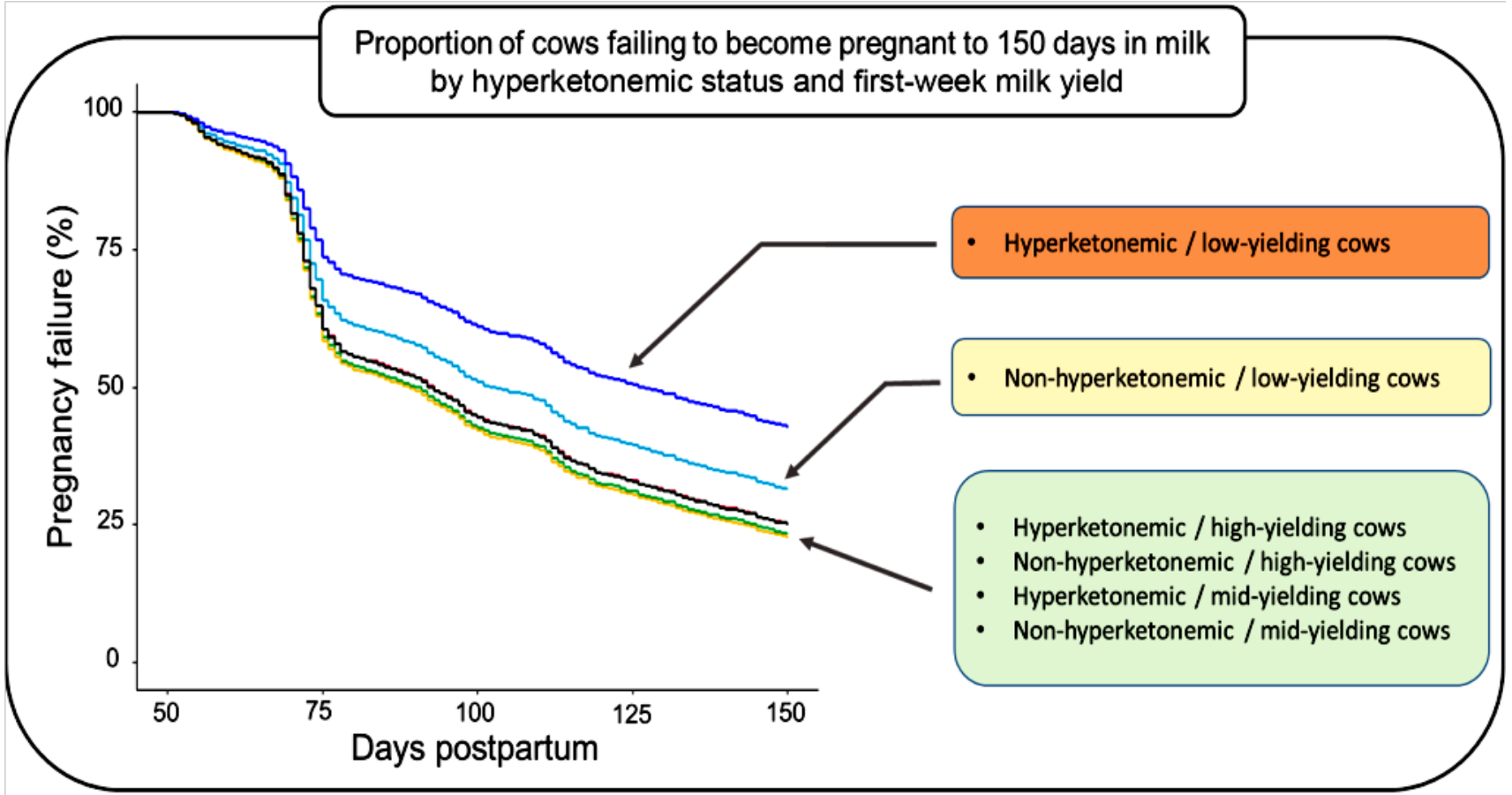
Animal Scientist Perspective:

- Not a disease but a normal metabolic response to support lactation
- No negative effects of BHB that exacerbate the problem
- Immune activation results in anorexia and leads to more severe hyperketonemia
- Is there an upper limit to ketone concentration?

Veterinarian Perspective:

- Ketosis is a disease associated with impaired health, fertility and production
- BHB above a threshold (subjective) considered abnormal
- Prevention or treatment reduces impact on performance - research
- Moderate heritability
- Farm-based disease risks indicating differences in management

Hyperketonemia, Milk Yield and Reproduction



- Hyperketonemia alone is not associated reproductive performance
- Low milk yielding cows with or without hyperketonemia had greater pregnancy failure

Rodriguez et al., JDS Comm 3 (2022)

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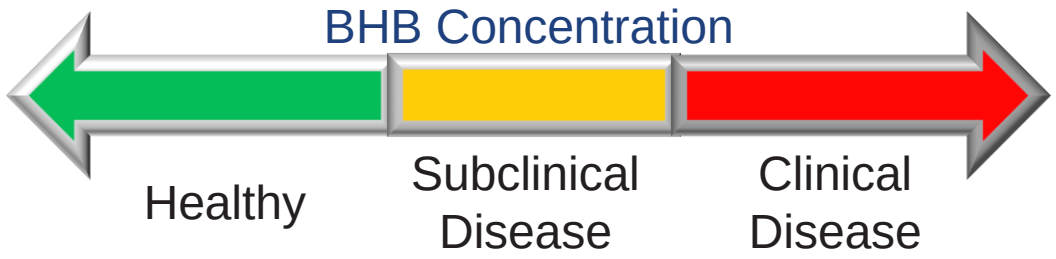
Fresh Cow Disease Risk – BHB Thresholds

Criteria	OR	95% CI	P
10 mg/dL >0.96 mmol/L	2.8	1.2 – 6.4	.01
12 mg/dL >1.15 mmol/L	3.5	1.4 – 8.5	.005
14 mg/dL >1.34 mmol/L	4.2	1.5 – 11.5	.004
16 mg/dL >1.54 mmol/L	5.6	1.8 – 18.0	.002
18 mg/dL >1.74 mmol/L	6.5	1.8 – 23.9	.002
20 mg/dL >1.92 mmol/L	5.0	1.3 – 18.6	.01

Van Saun, 2004

- Associations between blood BHB concentration and disease are not cause and effect
- Increasing disease risk with increasing BHB concentration suggests some connection
- Elevated BHB reflects a state of abnormal metabolism



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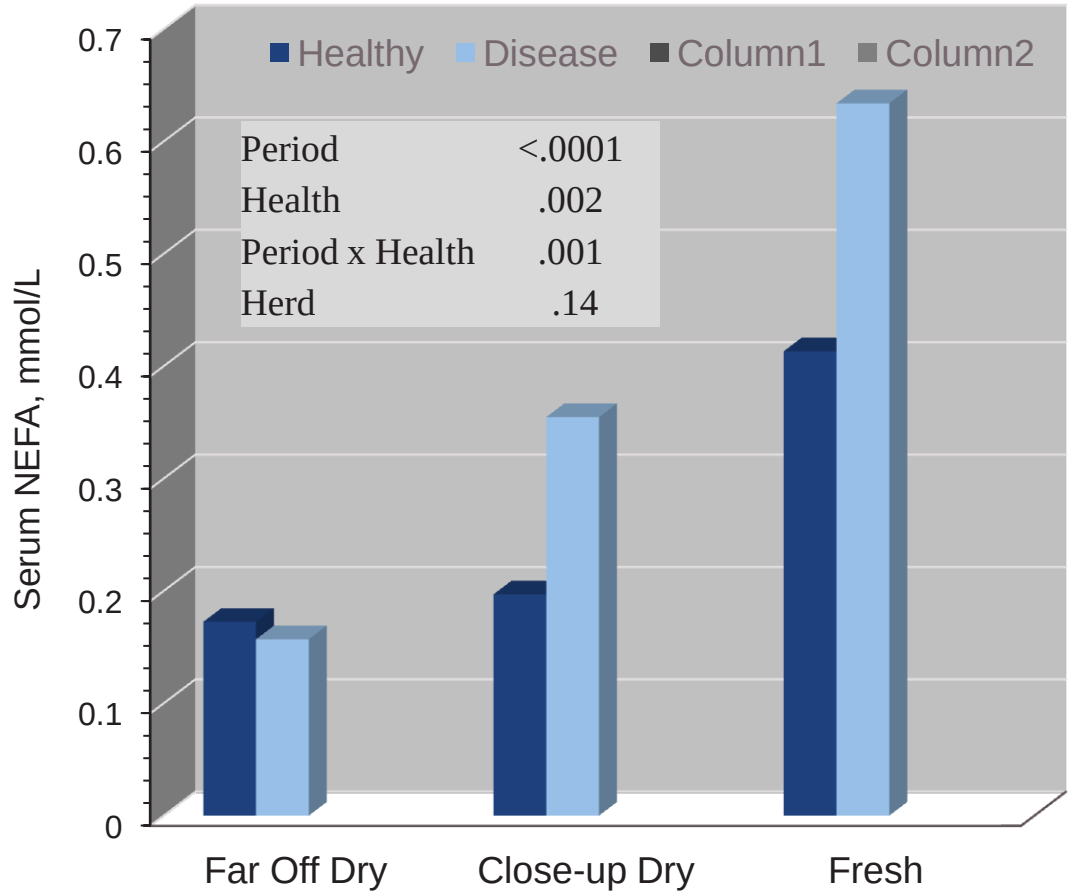
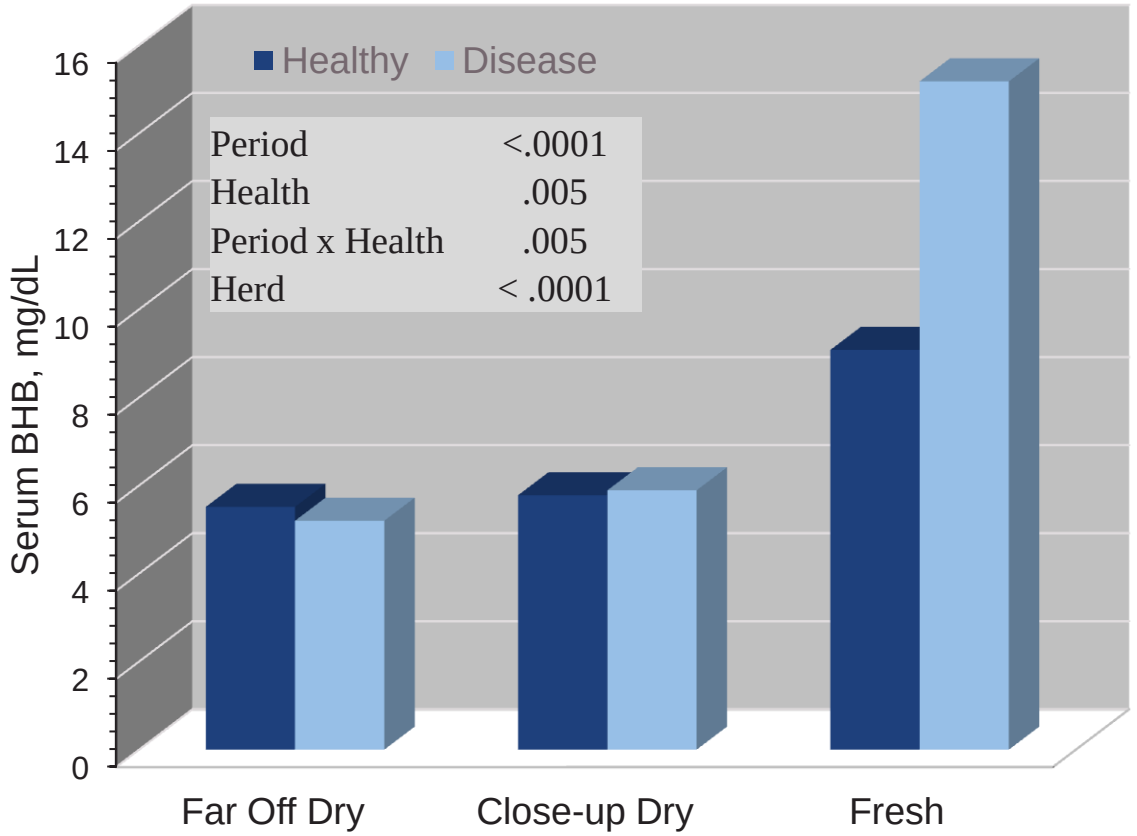
NEFA and BHB Concentrations in Transition Cows





Van Saun, 2004

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Fresh Cow Metabolites – Ketosis Categories

	Normal		Subclinical		Clinical	
Blood Metabolite	**≤ 1.4 mmol/L**		**> 1.4 & ≤ 2.6 mmol/L**		**> 2.6 mmol/L**	
	Healthy	**Sick**	**Healthy**	**Sick**	**Healthy**	**Sick**
BHB, mmol/L	**0.58**	**0.56**	**1.65**	**1.87**	**3.74**	**3.74**
Albumin, g/L[1]	**34.4**	**34.2**	**34.3**	**32.4**	**38.1**	**32.0**
Glucose, mmol/L[2]	**3.30**	**3.48**	**2.82**	**2.38**	**1.94**	**1.93**
Cholesterol, mmol/L[3]	**3.26**	**3.30**	**4.99**	**3.10**	**4.20**	**2.57**
NEFA, mmol/L[4]	**0.284**	**0.313**	**0.524**	**0.871**	**0.519**	**1.13**

[1]Health, P<.01, BHB x Health, P<.06

[2]BHB, P<.0001; BHB x Health, P<.09

[3]BHB, P<.01; Health, P<.005; BHB x Health, P<.0003

[4]BHB, P<.0001; Health, P<.006; BHB x Health, P<.02

Van Saun, 2004

Fresh Cow Metabolites – BHB Categories

	Normal		Moderate		Elevated	
Blood Metabolite	**≤ 1.0 mmol/L**		**> 1.0 & ≤ 2.0 mmol/L**		**> 2.0 mmol/L**	
	Healthy	**Sick**	**Healthy**	**Sick**	**Healthy**	**Sick**
BHB, mmol/L	**0.636**	**0.61**	**1.24**	**1.45**	**2.62**	**3.11**
Albumin, g/L[1]	**34.5**	**34.7**	**35.4**	**32.2**	**35.4**	**32.3**
Glucose, mmol/L[2]	**2.99**	**3.04**	**3.36**	**2.46**	**2.33**	**2.00**
Cholesterol, mmol/L[3]	**3.59**	**3.80**	**4.49**	**3.54**	**3.54**	**3.54**
NEFA, mmol/L[4]	**0.436**	**0.365**	**0.477**	**0.648**	**0.712**	**0.994**

[1]Health, P<.06

[2]BHB, P<.01; Health, P<.07; BHB x Health, P<.04

[3]BHB x Health, P<.05

[4]BHB, P<.005

Van Saun, 2004

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Fresh Cow Metabolites – NEFA Status

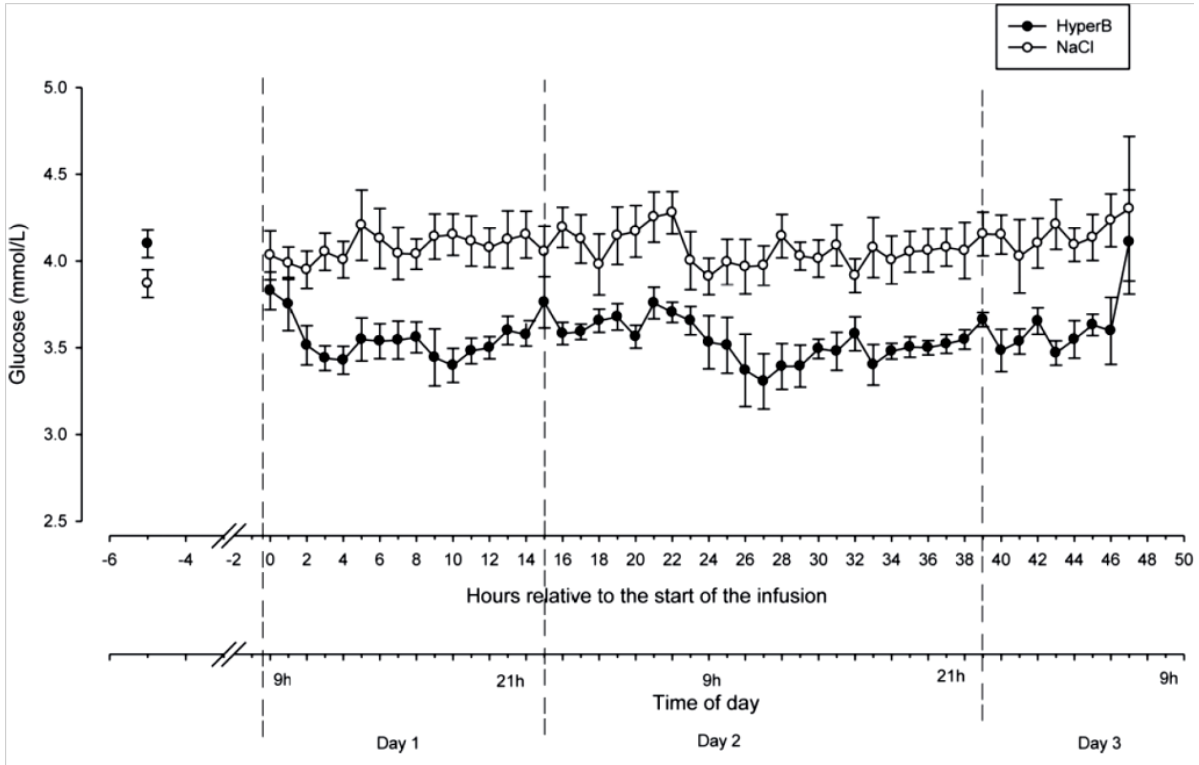
Blood Metabolite	< 0.6 mmol/L		≥ 0.6 mmol/L	
	Healthy	Sick	Healthy	Sick
NEFA, mmol/L	0.351	0.308	0.948	0.982
Albumin, g/L	34.9	33.2	34.8	33.5
Glucose, mmol/L[1]	2.90	2.78	3.68	2.35
Cholesterol, mmol/L	3.79	3.72	3.53	3.51
BHB, mmol/L[2]	0.84	1.01	0.81	2.15

[1]Health, P<.002; NEFA x Health, P<.001

[2]NEFA, P<.01; Health, P<.001; NEFA x Health, P<.01

Van Saun, 2004

BHB Infusion Effects



- Study performed IV BHB infusion over 48 hours
- Blood BHB achieved 1.7 mmol/L
- No effect on DMI, milk yield or energy balance
- Decline in blood glucose concentration – potentially due to reduced gluconeogenesis and lower glucagon
- No impact on insulin or enzymes of energy metabolism
- BHB had “glucose sparing” effect

Zarrin et al., J Dairy Sci 96 (2013)

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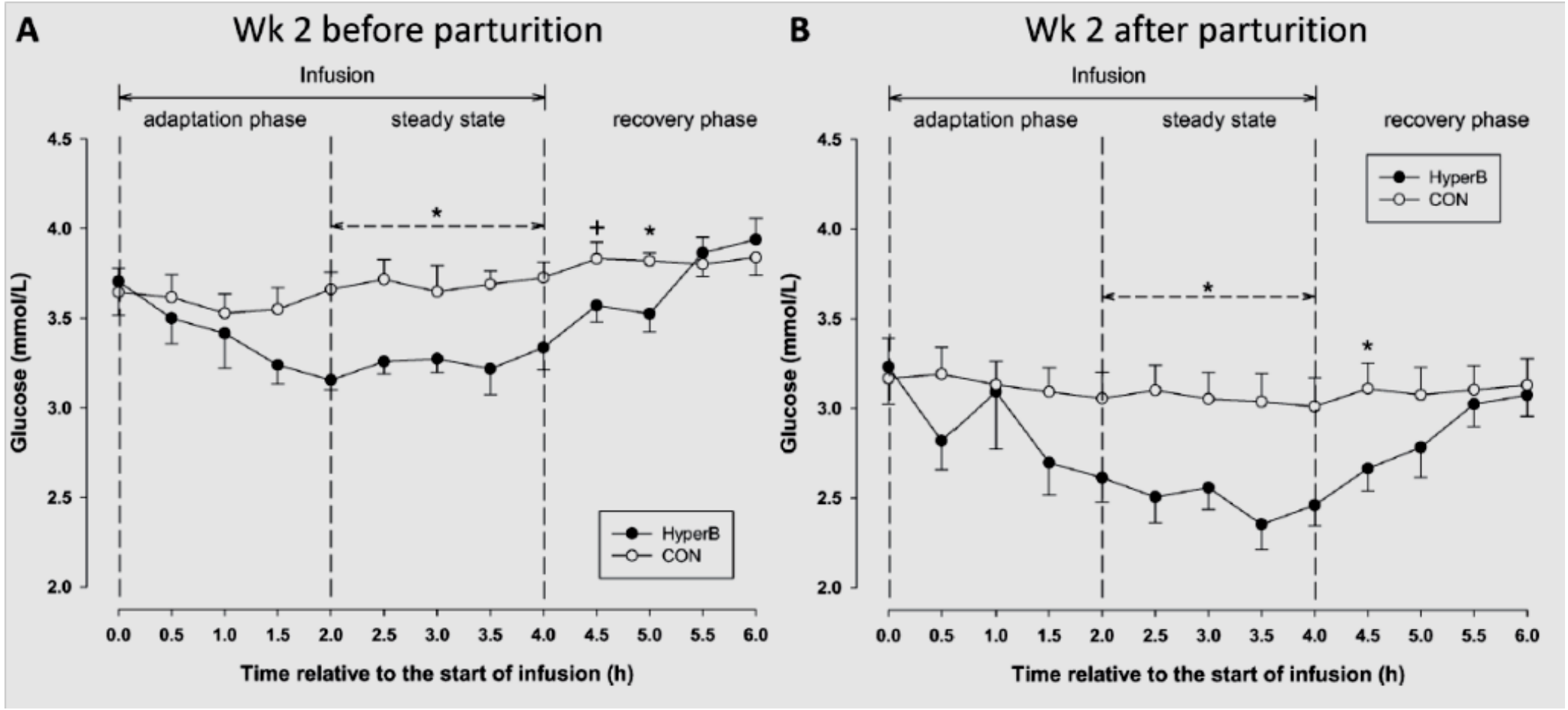
BHB Infusion Effects



Zarrin et. al., J Dairy Sci 100 (2017)

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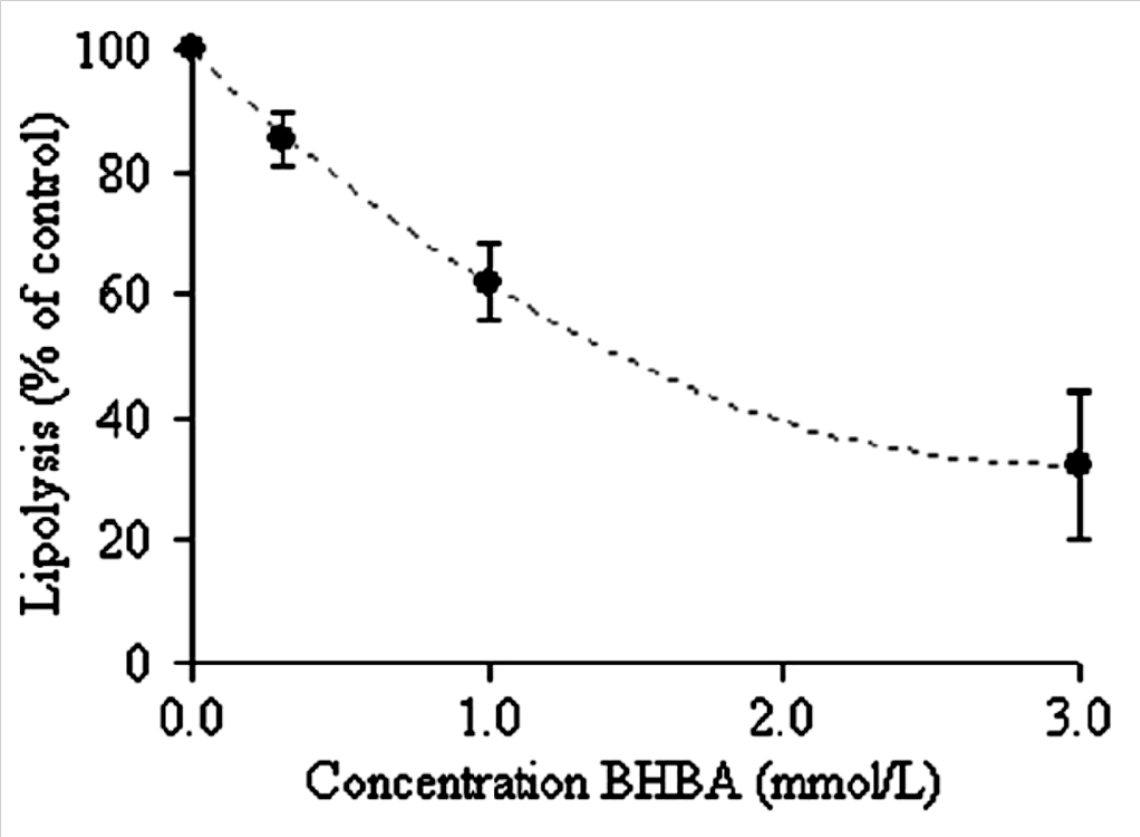
Inhibition of Lipolysis



- In vitro study of adipose cells responsiveness to BHB or β-adrenergic agonist
- Inhibition of lipolysis by BHB was greater in late gestation compared to early lactation
- BHB reduced lipolysis by 45% in adipocytes from cows with clinical ketosis
- Suggests BHB does not exacerbate lipolysis further contributing to ketosis

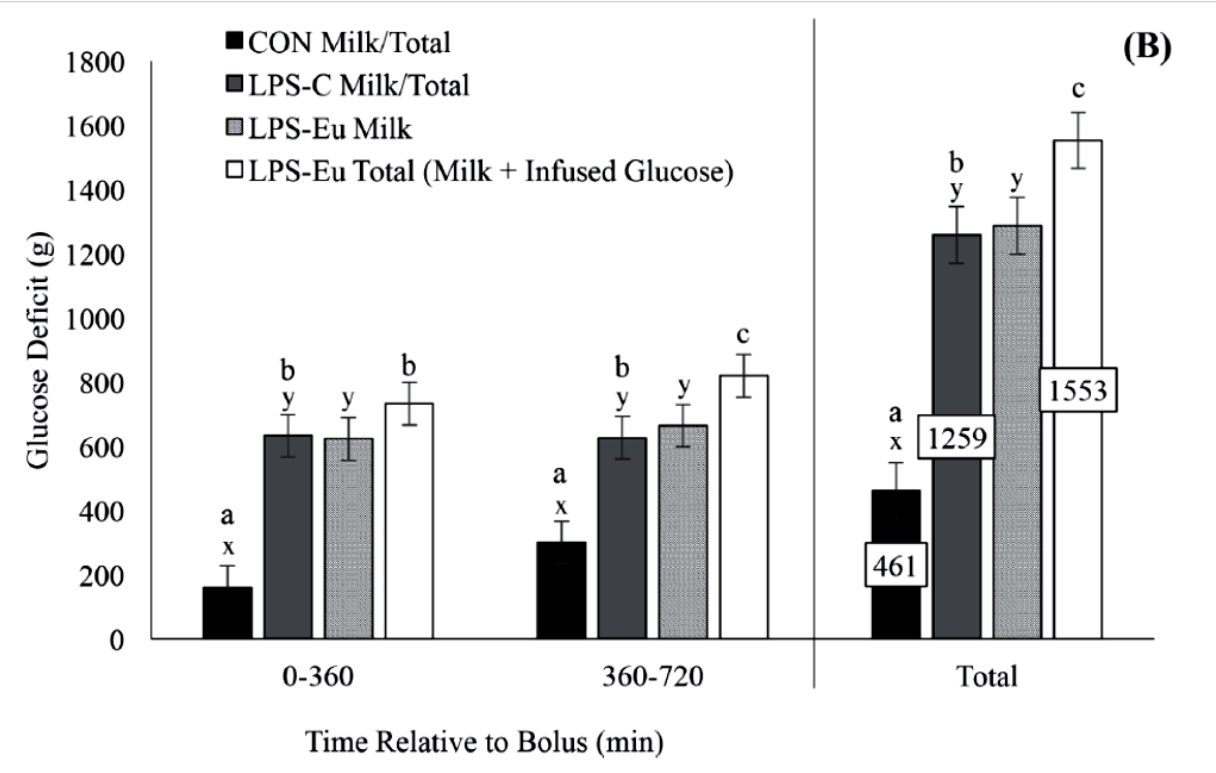
van der Drift et al., Res Vet Sci 94 (2013)

Immune Response

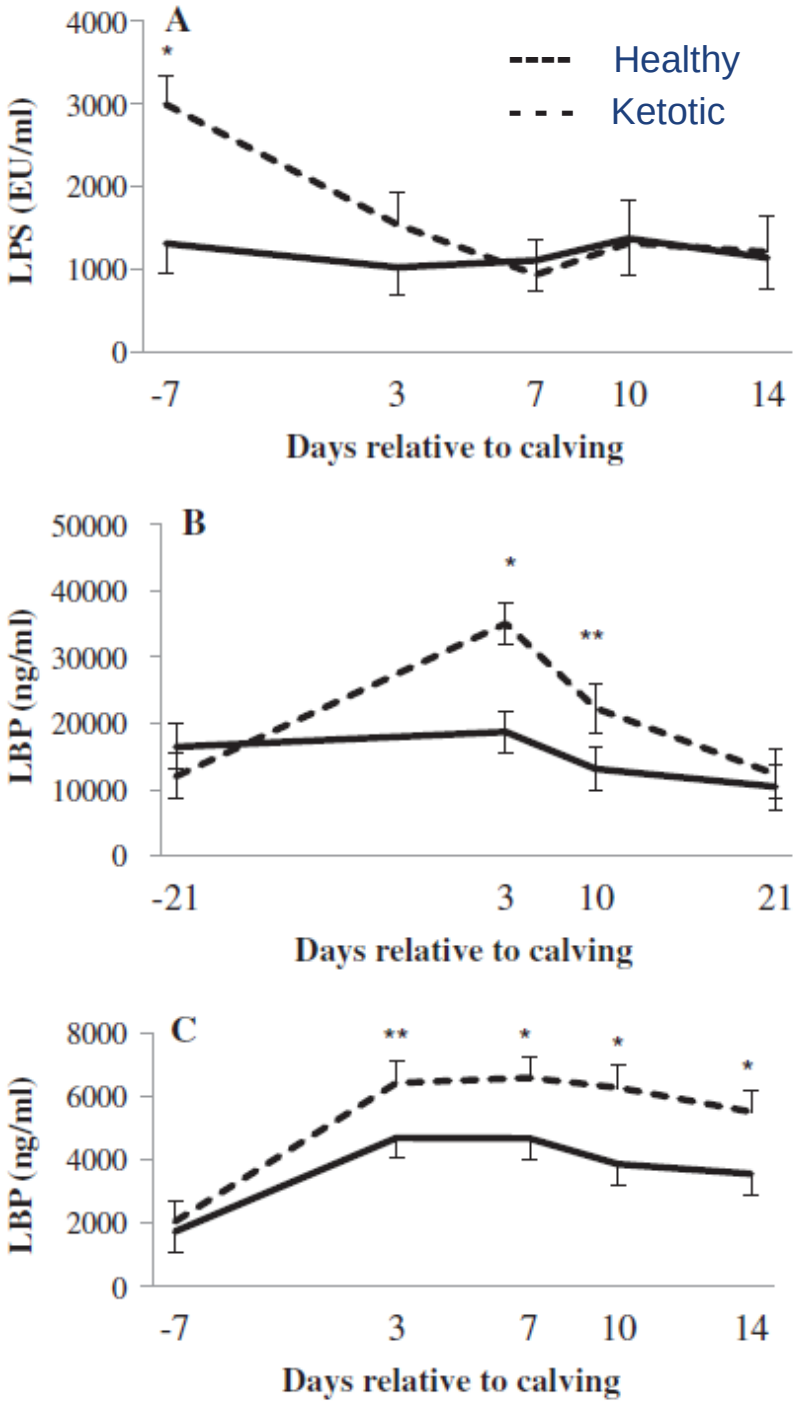
- Adverse effects on immune cells:
  - ↓ Respiratory burst of phagocytes
  - ↓ Killing capacity
  - ↓ Phagocytosis by neutrophils
  - ↓ Chemotaxis
  - ↓ Lymphocyte blastogenesis
  - ↓ IgM secretion

Ingvartsen and Moyes, Animal 7 (2012); Dänicke et al., Res Vet Sci 116 (2018); Zhang et. al., Res Vet Sci 107 (2016)

Increased glucose utilization during active inflammatory response. Immune system uses > 1 kg of glucose 12 hours.



Kvidera et al., J Dairy Sci 100 (2017)



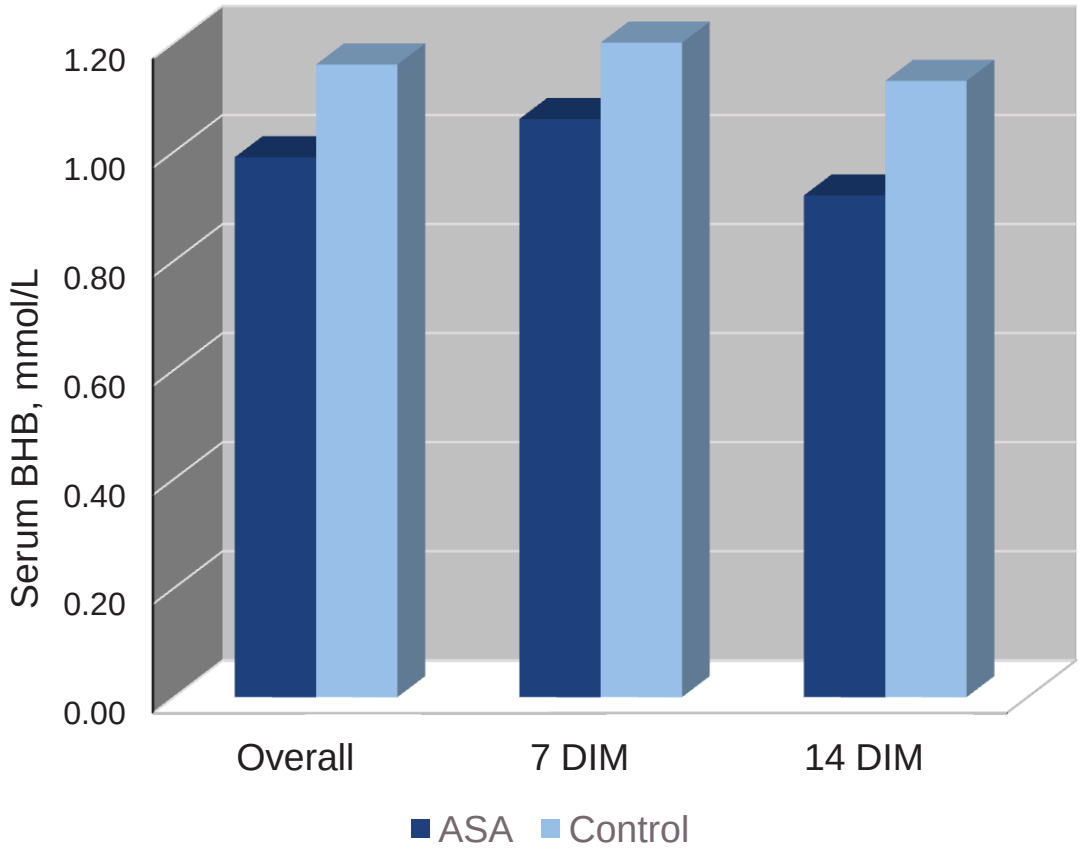
Inflammation and Ketosis

- Increase in circulating inflammatory markers identified pre- and postpartum in ketotic cows
- Source of inflammation was not identified in the study – potentially "leaky gut"
- Proinflammatory mediators are potent suppressors of intake
- Activate inflammatory process is nutritionally costly, consuming both glucose and amino acids

Abuajamieh et al., Res Vet Sci 109 (2016)

Stressor Factors and Inflammation

- A range of environmental, social, or management factors may contribute to an inflammatory response
- Overcrowding of transition pens
- Social order upheaval
  - Parity groups
  - Pen changes
- Heat stress
- Feed availability
  - Bunk space



Barragan et al., J Dairy Sci 103 (2020)

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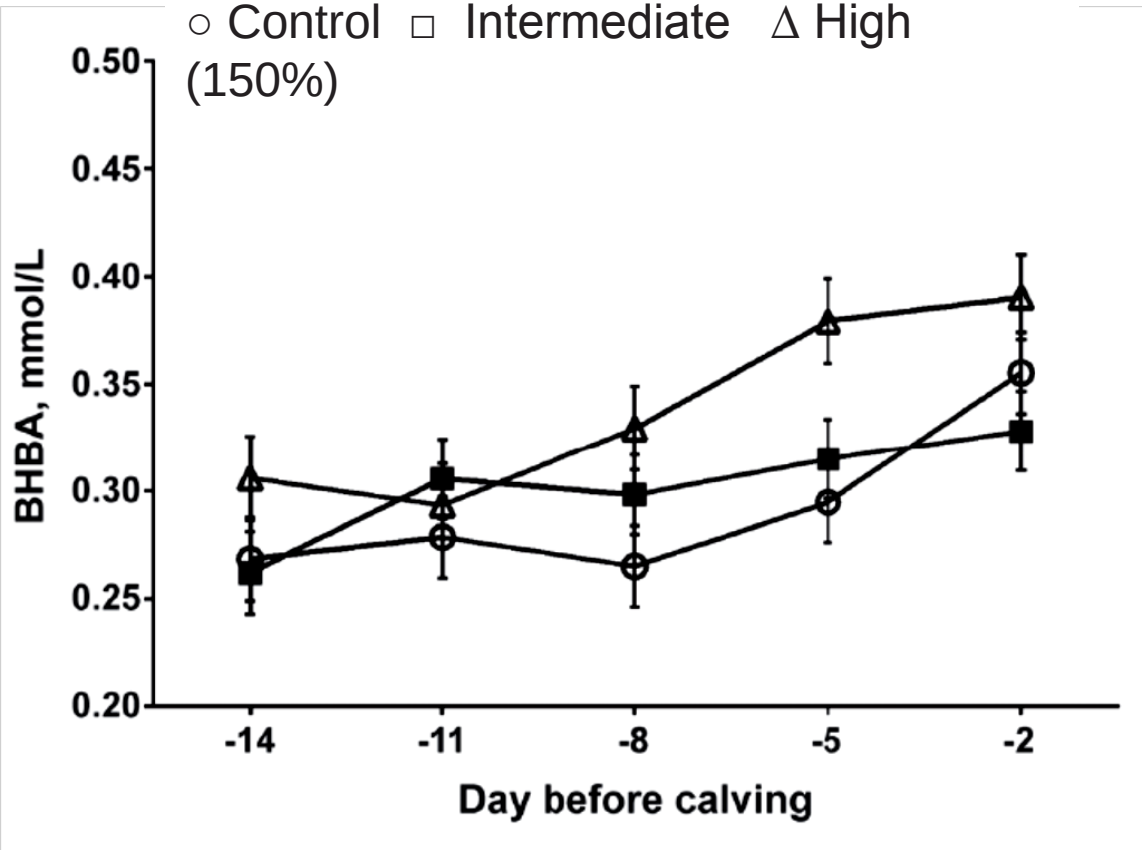
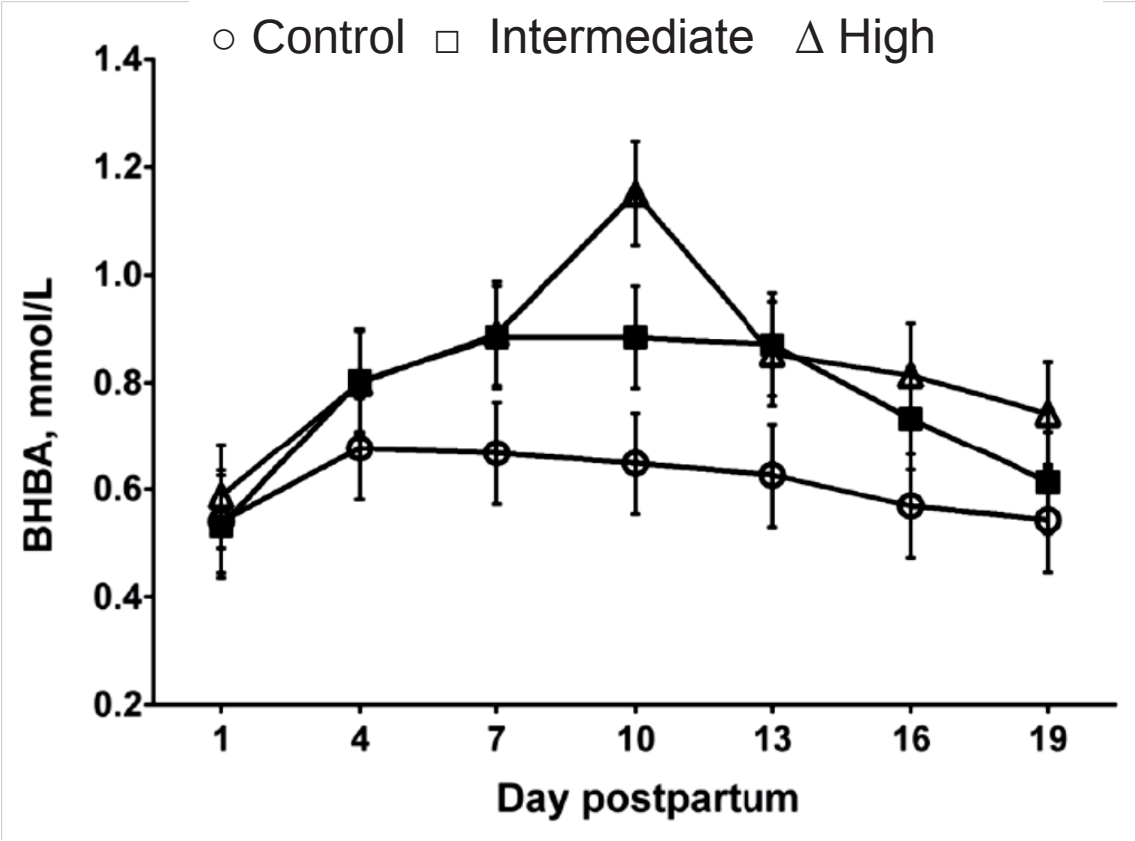
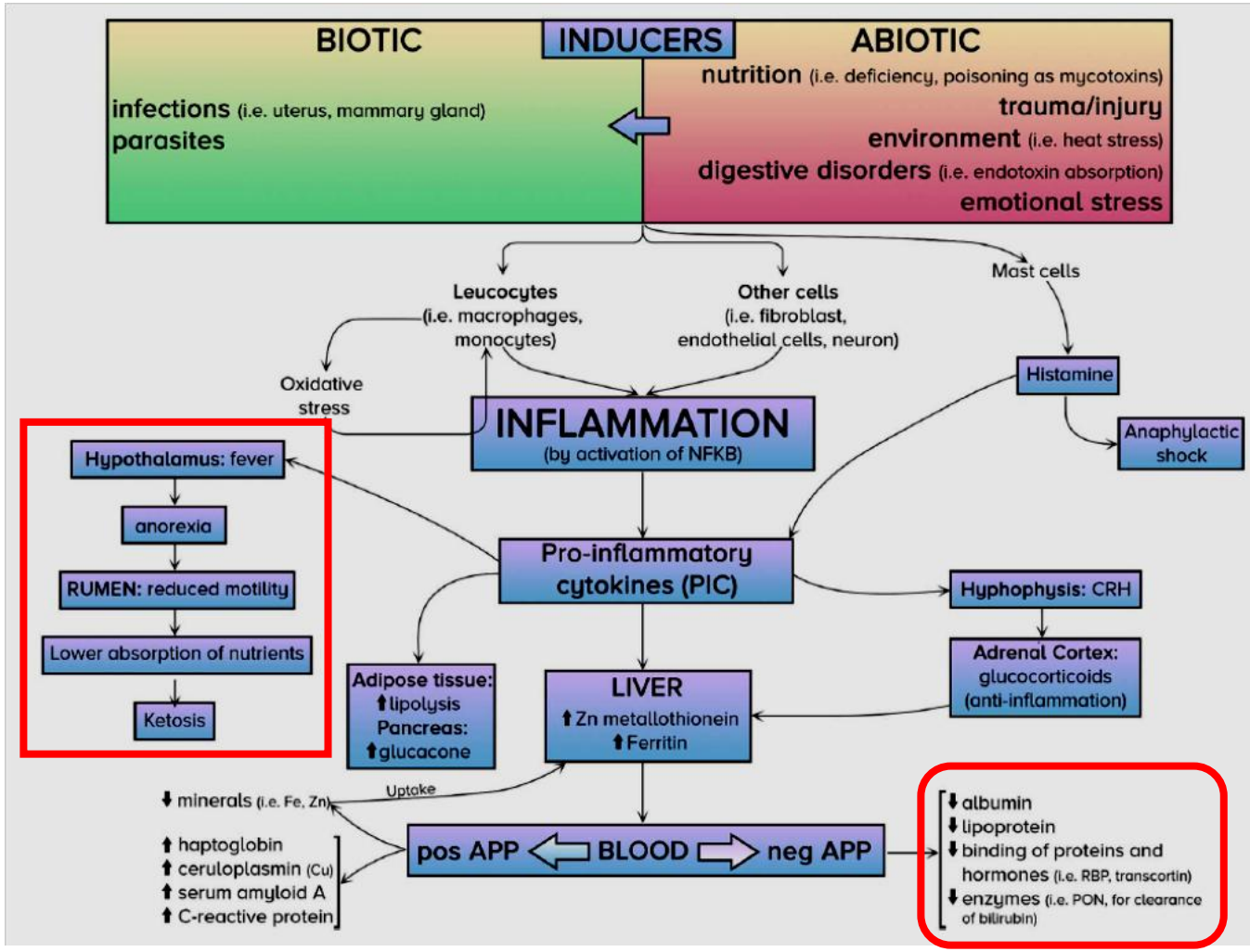
Dietary Energy Effects on BHB

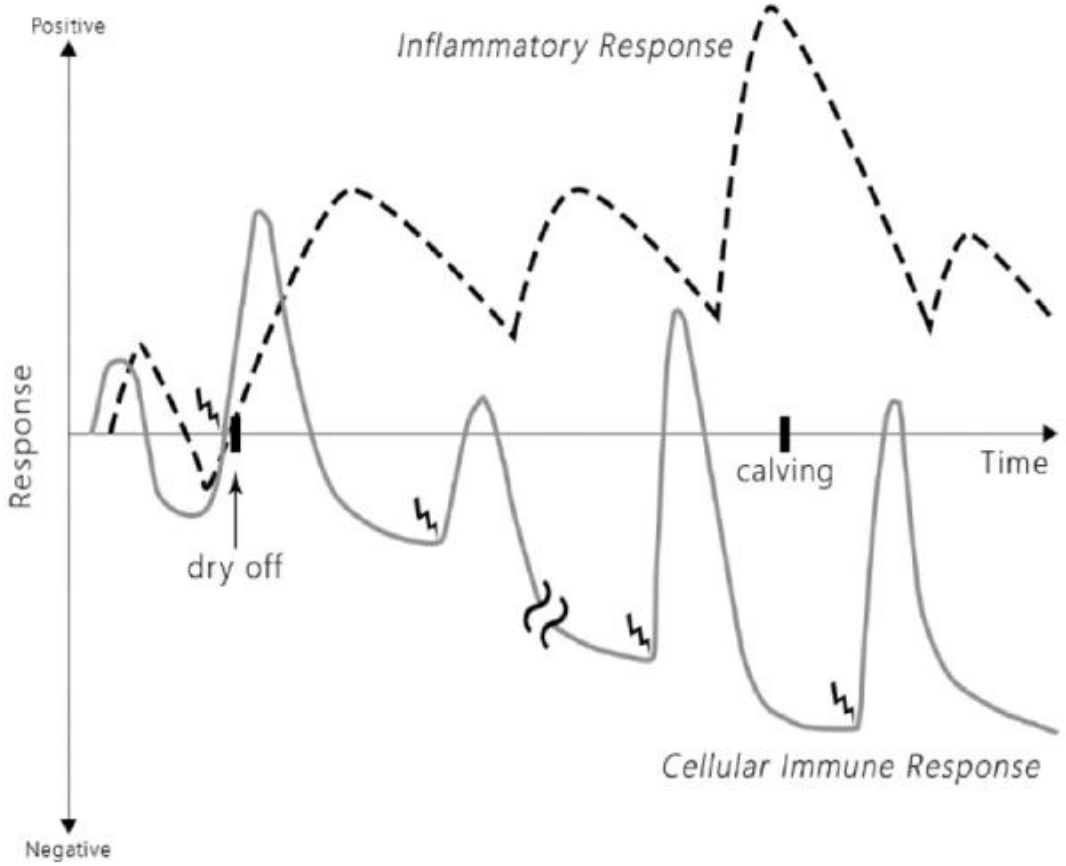




Mann et al., J Dairy Sci 98 (2015)

Cyclic Inflammation and Immunity Effects





Trevisi and Minuti, Res Vet Sci 116 (2018)

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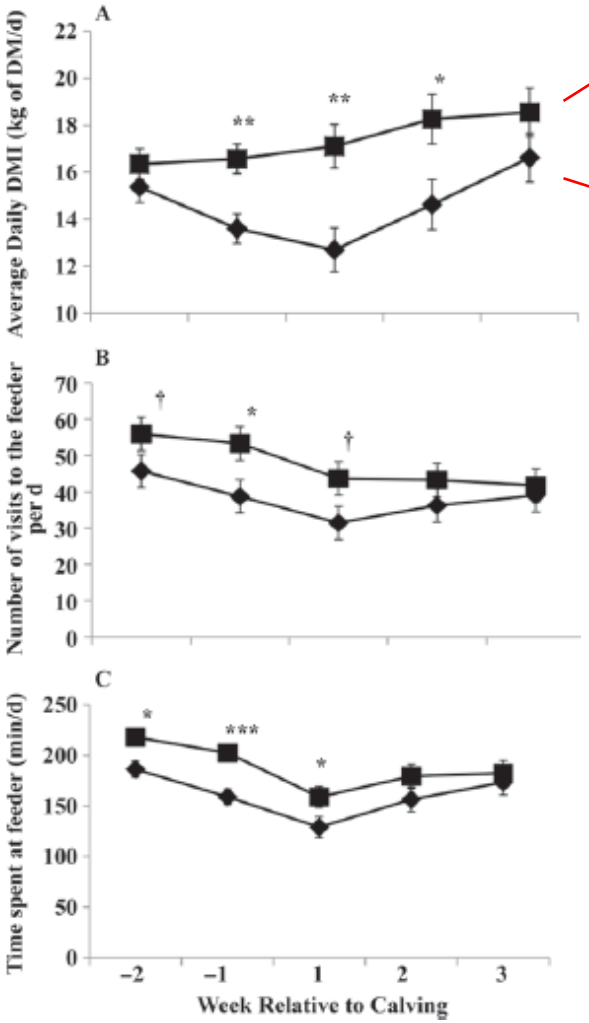
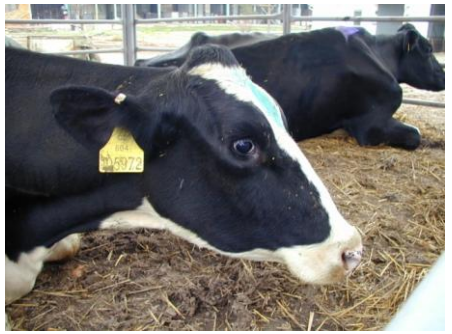
Dry Matter Intake PP and Ketosis

Dry matter intake and feeding time by healthy and ketotic cows



healthy



ketotic

Ketotic cows consumed less dry matter than healthy cows 1-2 weeks before diagnosis.
They also spend less time eating

Goldhawk et al., 2009

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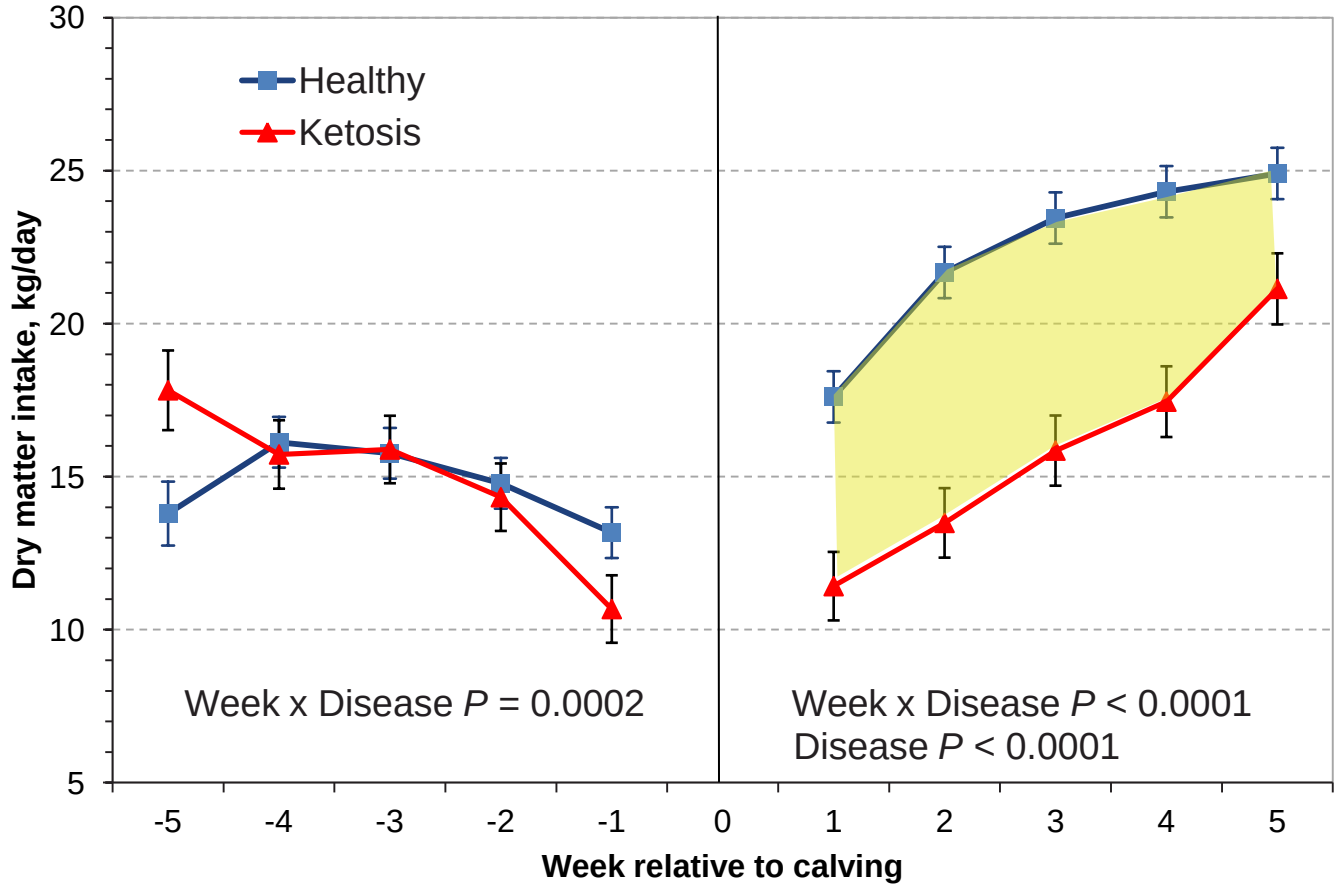
Dry Matter Intake and Ketosis



- Total DMI loss in first 5 weeks was 228 kg
- Using dietary energy content, total energy loss would account for:
  - 0.88 Body Condition Score loss
  - 470 kg 4% milk yield loss
- What is causing the prepartum intake drop?

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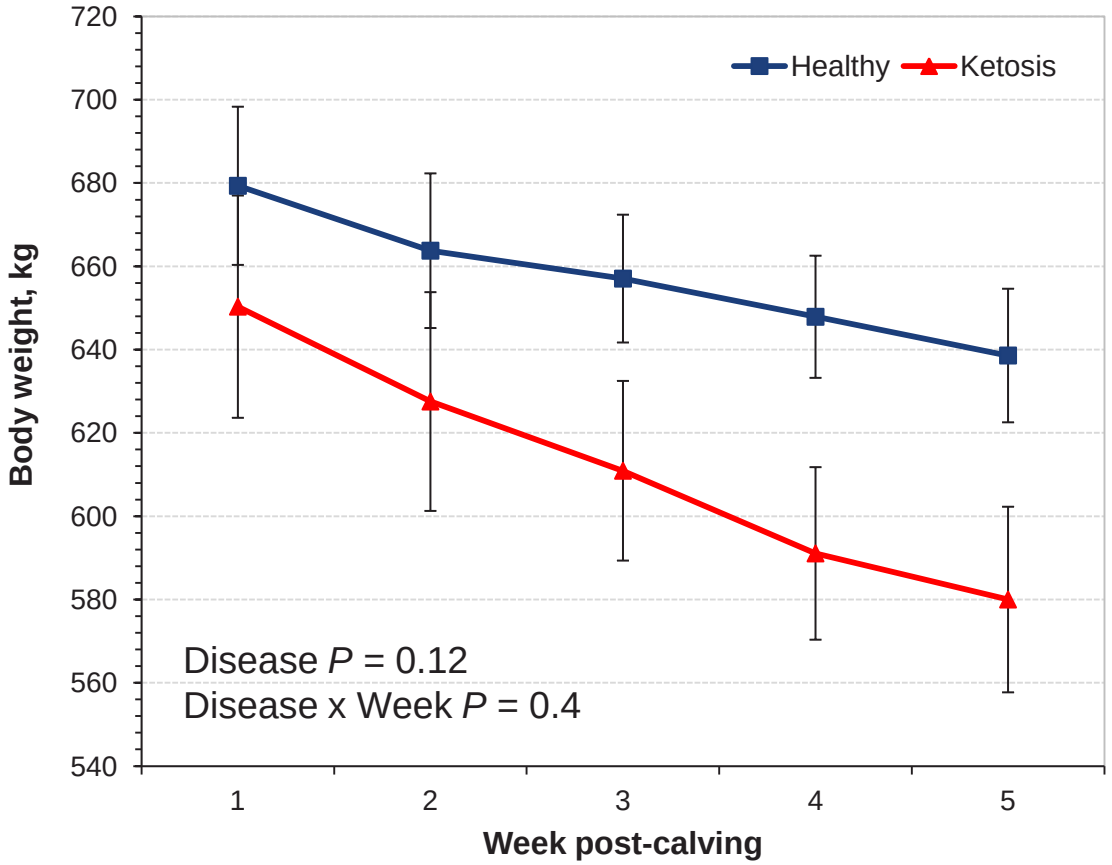
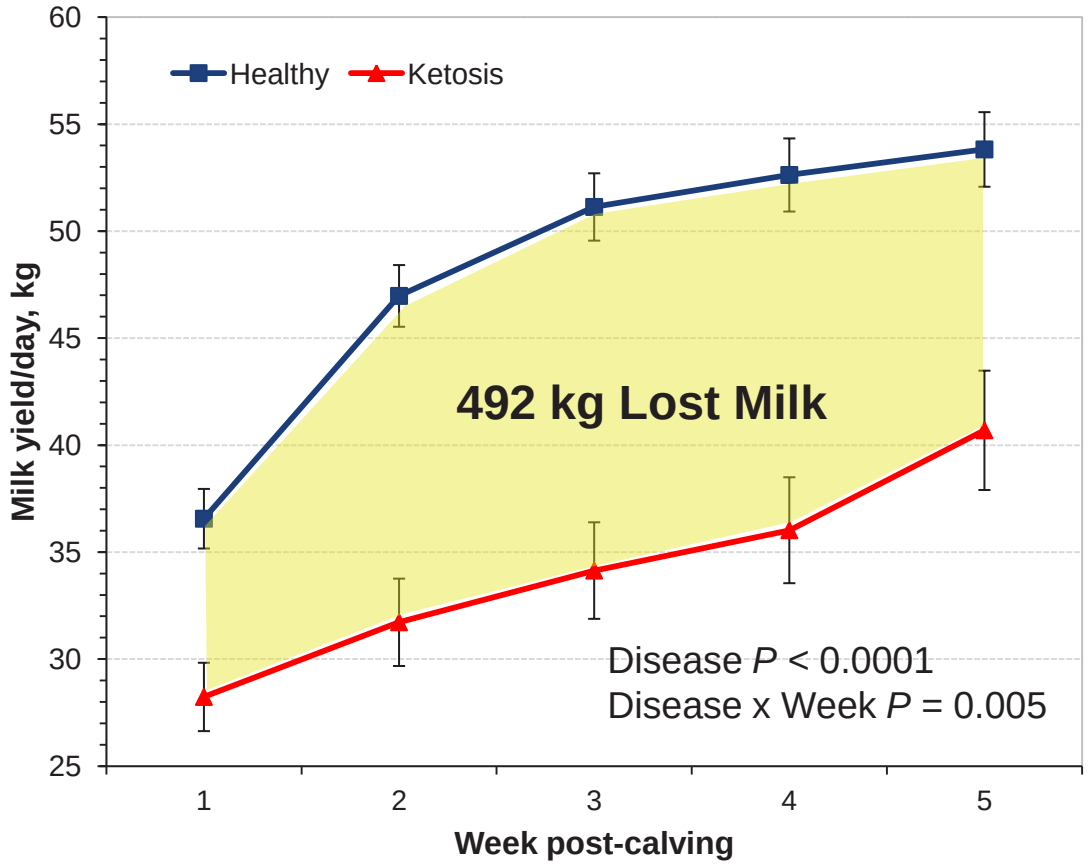
Ketosis – Postpartum Performance





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Take Home Points

- Ketone body production is an essential metabolic adaptation to initiation of lactation to address the period of negative energy balance
- Pathogenesis of ketosis is more complex than dysregulation of glucose homeostasis and elevated BHB concentration (other ketone bodies- acetone?)
- Effects of elevated BHB cannot account for observed clinical signs of ketosis, but it does compromise immune function
- Active inflammation can account for reduced feed intake, glucose and amino acid consumption potentially exacerbating metabolic adaptations in transition leading to ketosis

Take Home Points

- Ketosis prevention should start with body condition score management in late lactation to prevent over conditioning
- Dry cow diet should provide energy needs without exceeding requirement and sufficient metabolizable protein
- Marked increases in dietary starch during transition should be avoided
- Management should focus on minimizing potential stressors through the dry and early lactation period to minimize inflammatory activation

Thank you!

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Questions?

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