Epi 101

Cause and consequence

Understanding and applying information from different types of studies of dairy cow health and performance

1

What do you want to know?

Socrates' questions	Scientist's questions	Dairy advisor's & manager's questions
• Is it true? • Is it good? • Is it useful?	• What is the mechanism? • What is the physiological relevance?	• Is profitable or helpful to my operation to do this?

2

Types of Study Designs:

Descriptive – no comparison

- Case Report
- Case Series
- Survey

These are relatively easier to do and a great place to start, but offer little "proof", and may or may not be related to your world

3

Types of Study Designs:

Analytic – have a comparison group

- Observational
  - Cross-sectional
  - Cohort
  - Case-control
- Experimental
  - Laboratory
  - Controlled Trials

Differences in when exposure is measured in relation to outcome

Differences in how study subjects are allocated into groups

4

What are you talking about?

- Hyperketonemia = elevated blood concentration of ketones, generally BHB
  - Ketonemia - A biomarker of homeorhetic adaptation to lactation, implying lipolysis and ketogenesis as an alternative fuel to spare glucose for lactogenesis
  - Hyperketonemia (HYK) - A biomarker of maladaptation; a threshold associated with greater risk of undesirable outcomes
    - DA
    - Uneconomic/premature culling
    - Achievement and timing of pregnancy
    - Milk yield
- Clinical ketosis – a visible disease state with signs including inappetence concurrent with HYK; in the extreme form, neurologic signs
  - **There is no threshold of BHB consistently associated with clinical signs**
- Subclinical ketosis – (rightly or not) used interchangeably with HYK; no visible signs of disease

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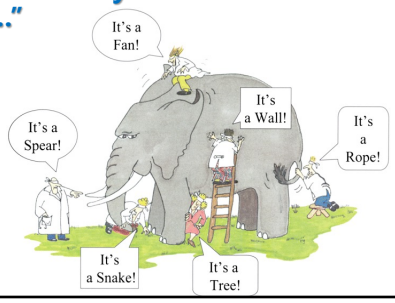
Are elevated circulating ketones a problem worth time and money? "It depends…"



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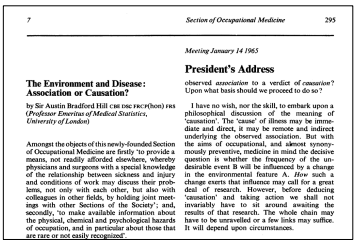
How do we know what causes what?



7 Section of Occupational Medicine 295

Meeting January 14 1965

President's Address

The Environment and Disease: Association or Causation?

by Sir Austin Bradford Hill CBE DSC FRCP(HON) FRS (*Professor Emeritus of Medical Statistics, University of London*)

Amongst the objects of this newly-founded Section of Occupational Medicine are firstly 'to provide a means, not readily afforded elsewhere, whereby physicians and surgeons with a special knowledge of the relationship between sickness and injury and conditions of work may discuss their problems, not only with each other, but also with colleagues in other fields, by holding joint meetings with other Sections of the Society'; and, secondly, 'to make available information about the physical, chemical and psychological hazards of occupation, and in particular about those that are rare or not easily recognized'.

observed *association* to a verdict of *causation*? Upon what basis should we proceed to do so?

I have no wish, nor the skill, to embark upon a philosophical discussion of the meaning of 'causation'. The 'cause' of illness may be immediate and direct, it may be remote and indirect underlying the observed association. But with the aims of occupational, and almost synonymously preventive, medicine in mind the decisive question is whether the frequency of the undesirable event B will be influenced by a change in the environmental feature A. *How* such a change exerts that influence may call for a great deal of research. However, before deducing 'causation' and taking action we shall not invariably have to sit around awaiting the results of that research. The whole chain may have to be unravelled or a few links may suffice. It will depend upon circumstances.

Bradford Hill Criteria

Proc Royal Society of Medicine, 1965

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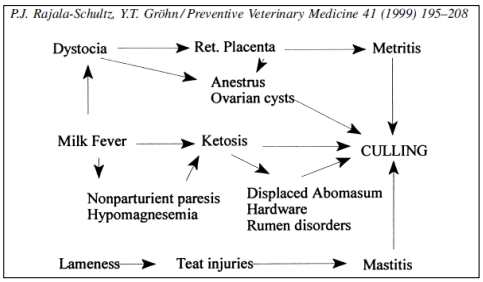
Classical associations

P.J. Rajala-Schultz, Y.T. Gröhn / Preventive Veterinary Medicine 41 (1999) 195–208



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Useful ≠ Causal

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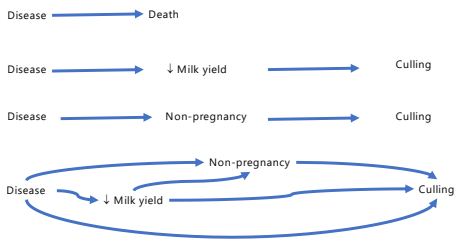
The relationships among health, production, fertility and survival are complex



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Survival bias

- A form of selection or inclusion bias
- Typically, data are only calculated on animals present at the time of measurement
  - Which cows are included in calculation of:
    - 305 day milk
    - Week 4 milk
    - Pregnancy at 1st AI
    - Pregnancy at 200 DIM
    - Age at 1st calving
    - 1st lactation milk yield

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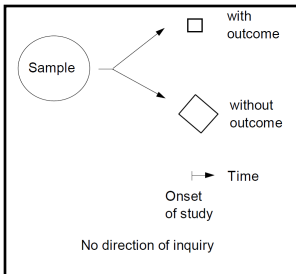

Dirk U. Pfeiffer

Professor of Veterinary Epidemiology

Q: What is happening?

Figure 25: Schematic diagram of cross-sectional study

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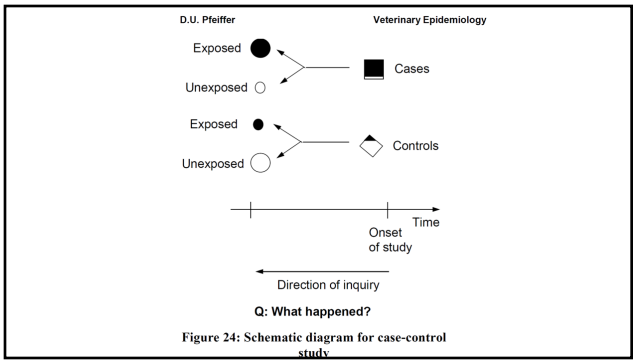

Figure 24: Schematic diagram for case-control study

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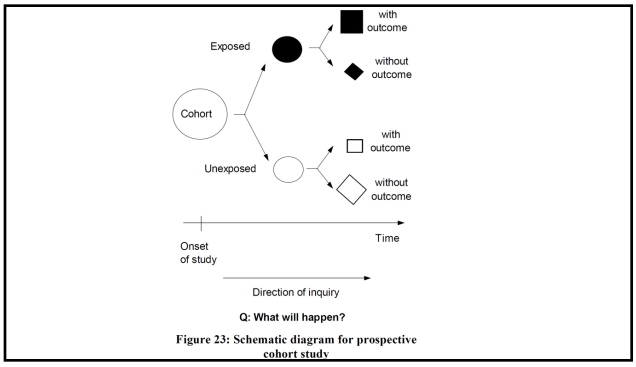

Figure 23: Schematic diagram for prospective cohort study

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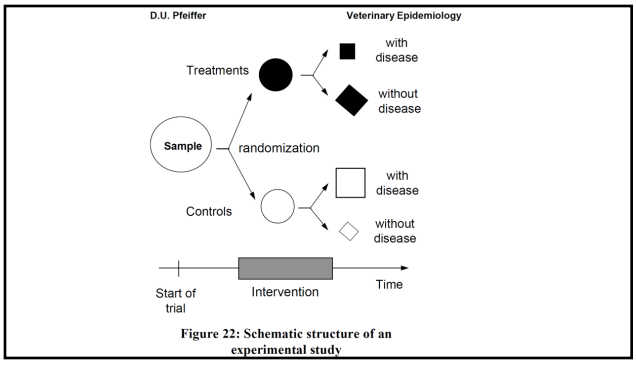

Figure 22: Schematic structure of an experimental study

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Criteria	Cross-sectional study	Case-control study	Prospective cohort study
Sampling	random sample of study population	separate samples of diseased and non-diseased units	separate samples of exposed and non-exposed units
Time	one point	usually retrospective	follow-up over specified period
Causality	association between disease and risk factor	preliminary causal hypothesis	causality through evidence of temporality
Risk	prevalence	none	incidence density, cumulative incidence
Comparison of risks	relative risk, odds ratio	odds ratio	relative risk, odds ratio

Figure 26: Comparison of observational field studies

Bias?:
Selection
Information
Confounding

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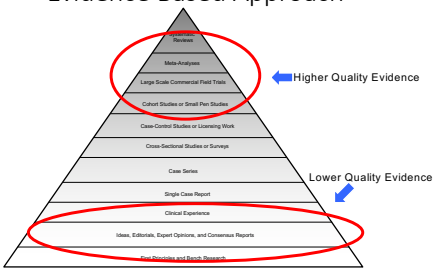
Evidence Based Approach



Adapted from Figure 4 – Relative strengths of evidence provided by different methods used in clinical research illustrated diagrammatically in the so-called pyramid of evidence. Strength of association increases from the base to the peak of the pyramid.

JAVMA, Vol 235, No. 9, November 1, 2009

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Does smoking cause cancer?

- …or, is smoking associated with cancer risk?
- There will never be a large-scale randomized controlled trial of the effect of smoking on the incidence of lung cancer
- There will never be an experimental challenge (in humans) of each of the chemicals in cigarette smoke to isolate which one(s) causes cancer
- Now do you want a light?

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Hazardous journeys

Parachute use to prevent death and major trauma related to gravitational challenge: systematic review of randomised controlled trials

Gordon C S Smith, Jill P Pell

Abstract

Objectives To determine whether parachutes are effective in preventing major trauma related to gravitational challenge.
Design Systematic review of randomised controlled trials.
Data sources: Medline, Web of Science, Embase, and the Cochrane Library databases; appropriate internet sites and citation lists.
Study selection: Studies showing the effects of using a parachute during free fall.
Main outcome measure Death or major trauma, defined as an injury severity score >15.
Results We were unable to identify any randomised controlled trials of parachute intervention.
Conclusions As with many interventions intended to prevent ill health, the effectiveness of parachutes has not been subjected to rigorous evaluation by using randomised controlled trials. Advocates of evidence based medicine have criticised the adoption of interventions evaluated by using only observational data. We think that everyone might benefit if the most radical protagonists of evidence based medicine organised and participated in a double blind, randomised, placebo controlled, crossover trial of the parachute.

accepted intervention was a fabric device, secured by strings to a harness worn by the participant and released (either automatically or manually) during free fall with the purpose of limiting the rate of descent. We excluded studies that had no control group.

Definition of outcomes
The major outcomes studied were death or major trauma, defined as an injury severity score greater than 15.[7]

Meta-analysis
Our statistical approach was to assess outcomes in parachute and control groups by odds ratios and quantified the precision of estimates by 95% confidence intervals. We chose the Mantel-Haenszel test to assess heterogeneity, and sensitivity and subgroup analyses and fixed effects weighted regression techniques to explore causes of heterogeneity. We selected a funnel plot to assess publication bias visually and Egger's and Begg's tests to test it quantitatively. Stata software, version 7.0, was the tool for all statistical analyses.

Results

Our search strategy did not find any randomised controlled trials of the parachute.

What is already known about this topic

Parachutes are widely used to prevent death and major injury after gravitational challenge

Parachute use is associated with adverse effects due to failure of the intervention and iatrogenic injury

Studies of free fall do not show 100% mortality

What this study adds

No randomised controlled trials of parachute use have been undertaken

The basis for parachute use is purely observational, and its apparent efficacy could potentially be explained by a "healthy cohort" effect

Individuals who insist that all interventions need to be validated by a randomised controlled trial need to come down to earth with a bump

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RESEARCH

OPEN ACCESS

Parachute use to prevent death and major trauma when jumping from aircraft: randomized controlled trial

Robert W Yeh,[1] Linda R Valsdottir,[1] Michael W Yeh,[2] Changyu Shen,[1] Daniel B Kramer,[1] Jordan B Strom,[1] Eric A Secemsky,[1] Joanne L Healy,[3] Robert M Domeier,[4] Dhruv S Kazi,[1] Brahmajee K Nallamothu[4] On behalf of the PARACHUTE Investigators

ABSTRACT

OBJECTIVE
To determine if using a parachute prevents death or major traumatic injury when jumping from an aircraft.

DESIGN
Randomized controlled trial.

SETTING
Private or commercial aircraft between September 2017 and August 2018.

PARTICIPANTS
92 aircraft passengers aged 18 and over were screened for participation. 23 agreed to be enrolled and were randomized.

INTERVENTION
Jumping from an aircraft (airplane or helicopter) with a parachute versus an empty backpack (unblinded).

MAIN OUTCOME MEASURES
Composite of death or major traumatic injury (defined by an Injury Severity Score over 15) upon impact with the ground measured immediately after landing.

RESULTS
Parachute use did not significantly reduce death or major injury (0% for parachute v 0% for control; P=0.9). This finding was consistent across multiple subgroups. Compared with individuals screened but not enrolled, participants included in the study were on aircraft at significantly lower altitude (mean of 0.6 m for participants v mean of 9146 m for non-participants; P<0.001) and lower velocity (mean of 0 km/h v mean of 800 km/h; P<0.001).

regarding the effectiveness of an intervention exist in the community, randomized trials might selectively enroll individuals with a lower perceived likelihood of benefit, thus diminishing the applicability of the results to clinical practice.

Introduction
Parachutes are routinely used to prevent death or major traumatic injury among individuals jumping from aircraft. However, evidence supporting the efficacy of parachutes is weak and guideline recommendations for their use are principally based on biological plausibility and expert opinion.[1 2] Despite this widely held yet unsubstantiated belief of efficacy, many studies of parachutes have suggested injuries related to their use in both military and recreational settings,[3 4] and parachutist injuries are formally recognized in the World Health Organization's ICD-10 (international classification of diseases, 10th revision).[5] This could raise concerns for supporters of evidence-based medicine, because numerous medical interventions believed to be useful have ultimately failed to show efficacy when subjected to properly executed randomized clinical trials.[6 7]

Previous attempts to evaluate parachute use in a randomized setting have not been undertaken owing to both ethical and practical concerns. Lack of equipoise could inhibit recruitment of participants in such a trial. However, whether pre-existing beliefs about the efficacy of parachutes would, in fact, impair the enrolment of participants in a clinical trial has not

WHAT IS ALREADY KNOWN ON THIS TOPIC

Parachutes are routinely used to prevent death or major traumatic injury among individuals jumping from aircraft, but their efficacy is based primarily on biological plausibility and expert opinion

No randomized controlled trials of parachute use have yet been attempted, presumably owing to a lack of equipoise

WHAT THIS STUDY ADDS

This randomized trial of parachute use found no reduction in death or major injury compared with individuals jumping from aircraft with an empty backpack

Lack of enrolment of individuals at high risk could have influenced the results of the trial

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Validity

Internal
- Are the outcomes measured accurately?
- Selection or inclusion bias; information bias; confounding bias?

External (generalizability)
- Is the study population relevant/comparable to my interest?
- Single herd studies – sometimes the best/sometimes no relation
- In vitro, cell culture, gene expression, or lab animal model studies

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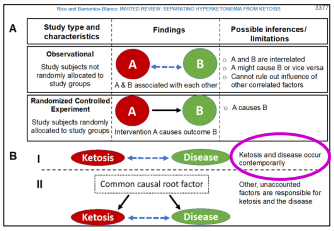
Possible misunderstandings of study design and interpretation



There is a longstanding debate in epidemiology about whether observational studies can establish causality.
They need not be to be useful.

RCT are not always feasible

Temporality can be established in observational studies

Confounders and unmeasured variables are a well-recognized problem that can be mitigated but never eliminated in any type of study

Rico & Barrientos-Blanco 2024

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Is my repro better or worse?

Or is my treatment protocol working?
i.e. comparing proportions

10%	95% CI	20%	95% CI
2/20	1.2 – 31.7	4/20	5.7 – 43.6
4/40	2.8 – 23.6	8/40	9.0 – 36.6
10/100	4.9 – 17.6	20/100	12.6 – 29.1
20/200	6.2 – 15.0	40/200	14.6 – 26.2
30/300	*6.8 – 13.9*	*60/300*	*15.6 – 24.9*
40/400	*7.2 – 13.3*	*80/400*	*16.1 – 24.0*
50/500	*7.7 – 12.9*	*100/500*	*16.5 – 23.4*

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Or is my treatment protocol working?
i.e. comparing proportions

70% Cures	90% CI	75% Cures	90% CI
70/100	61.5 – 77.3	3/4	24.8 – 98.7
140/200	64.2-75.3	30/40	61.3 – 85.7
280/400	66.0-73.7	300/400	71.1 – 78.5
420/600	*66.4 – 73.0*	*450/600*	*71.6 – 77.9*

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Confidence Interval

- 2000 milking cows, daily milk production 92 lbs, SD = 20
- 95% CI = 90 - 93
- 80% CI = 91 – 92.7

- 200 milking cows, daily milk production 92 lbs, SD=20
- 95% CI = 89 – 95
- 80% CI = 90 - 94

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The Width of the Confidence Interval
(the precision of the estimate)

The width of the confidence interval is affected by

- the confidence level ($1-\alpha$)
- the sample size (n).
- the population standard deviation (σ)
  - For continuous outcomes

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The American Statistician

ISSN: 0003-1305 (Print) 1537-2731 (Online) Journal homepage: http://www.tandfonline.com/loi/utas20

The ASA's statement on p-values: context, process, and purpose

Ronald L. Wasserstein & Nicole A. Lazar

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What is statistical significance?

- While the p-value can be a useful statistical measure, it is commonly misused and misinterpreted
- a p-value is the probability, under a specified statistical model, that a statistical summary of the data (e.g, the sample means in two compared groups) would be equal to or more extreme than its observed value

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A p-value, or statistical significance, does not measure the size of an effect or the importance of a result

- Statistical significance is not equivalent to scientific, human, or economic significance
- Smaller p-values do not necessarily imply the presence of larger or more important effects, and larger p-values do not imply a lack of importance or even lack of effect
- Any effect, no matter how tiny, can produce a small p-value if the sample size or measurement precision is high enough, and large effects may produce unimpressive p-values if the sample size is small or measurements are imprecise. Similarly, identical estimated effects will have different p-values if the precision of the estimates differs

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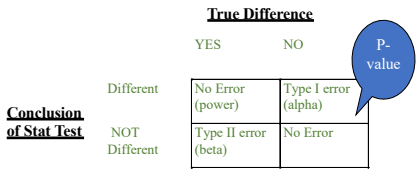
Results of Stats v. the "Truth"



Type 1 error: say there is a difference when there really isn't one

Type 2 error: say is NOT a difference when there really is one

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Which would you recommend?

- You ran a randomized controlled feeding trial in which you compared 2 feed additives (AD1 and AD2) to a baseline TMR. The baseline TMR was well formulated and delivered and the study conducted well, i.e. good internal validity.
- AD1 and AD2 were mixed well and included in the base TMR.
- Results:
  - AD1
    - 5lbs more FPCM/cow/day; increased DMI commensurate to support milk
    - P=0.07
    - no difference in rpro, SCC, GHG, or health disorders
    - Costs 5 cents/cow/day to add to diet.
  - AD2
    - 2lbs more FPCM/cow/day; increased DMI commensurate to support milk
    - P=.04
    - no difference in rpro, SCC, GHG, or health disorders
    - Costs 20 cents/cow/day to add to diet
- Assumed milk price_____; feed price_____

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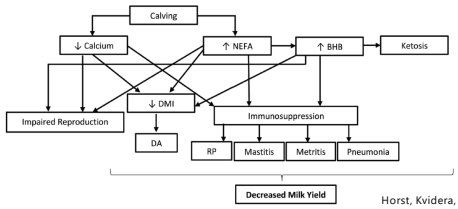
Who let the dogmas out? Association ≠ causation



Horst, Kvidera, Baumgard JDS 2021

https://www.youtube.com/watch?v=W0TMcVLznWk&list=PLbUWH8-a8MND_BtU1UktyvFr_359LtPh6&index=24

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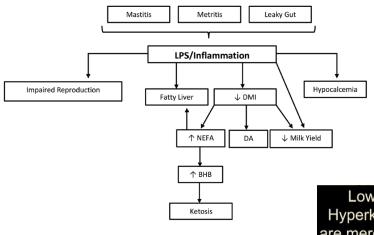
New hypothesis

Horst, Kvidera, Baumgard JDS 2021



Paradigm Shifting Theory

Increased NEFA and Hyperketonemia are caused by immune activation induced hypophagia

Hypocalcemia is a consequence of immune activation

Low Feed Intake, high NEFA, Hyperketonemia and hypocalcemia are merely SYMPTOMs….a reflection of prior immune stimulation

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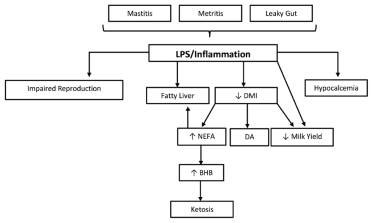
There are evidence and gaps for both hypotheses



Problems of temporality

↓ DMI before and at calving
↑ NEFA ± BHB often precede mastitis, metritis, DA

Little evidence of leaky gut in transition dairy cows

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Who let the dogmas out?

Critique

- Despite not using traditional intervening or controlled experimentation, increased NEFA, hyperketonemia, and hypocalcemia are presumed to have a causal relationship with poor transition cow success (Figure 1; Cameron et al., 1998; LeBlanc et al., 2005; Quiroz-Rocha et al., 2009; Ospina et al., 2010a; Chapinal et al., 2011; Huzzey et al., 2011).

Discussion

- Little indication in these studies of ascribing causality
- Possibly some contentious use of 'effect'

Cause → effect

Vs

Exposure → association

- Measures of association (observational) and measures of effect (intervention) use the same metrics: OR, RR, HR, risk difference, kg of milk, etc.

Horst et al., 2021

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Who let the dogmas out?

Critique

- Dozens of peer-reviewed articles have demonstrated an association between metabolites and transition cow problems, but importantly numerous inconsistencies exist.
- … these tenets are largely based on associations and not cause-and-effect relationships garnered from controlled and intervening experimentation.

Horst et al., 2021

Discussion

- Every study, including RCT, is a random sample of the 'truth'. Variability is expected.
- Associations with increased risk of disease and culling are quite consistent
- True for almost all health disorders in dairy cows.
- Experimental induction of health disorders is practically impossible at the scale to assess milk, repro, or culling outcomes.

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Who let the dogmas out?

Critique

- In addition, inconsistent association metrics (e.g., odds ratio, relative risk, hazard ratio) are used to assess the relationship.
- The conflicting relationships described above exemplify the dogma's limitations and highlight the boundaries of retrospective classification and epidemiology.

Horst et al., 2021

Discussion

- Different outcomes require different measures
  - Event yes or no → OR or RR
  - Time to event → HR
- Relative measures such as OR and RR should be accompanied by estimates of absolute measures e.g., % affected. This could be improved in many reports.
- Dogma is never helpful to science. Dogmatic insistence on a single type of evidence isn't either.

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Who let the dogmas out?

Critique

1. The nature of the relation of circulating ketones with production and health is inconsistent
2. Unresolved inflammation and high blood levels of lipotoxic free FA (FFA) typically ensue contemporaneously with—and can even precede—ketosis… excess FFA and unresolved inflammation can cause metabolic dysfunction, compromising production and health independently of ketones
3. Current treatments for alleviating ketosis have limited and variable effectiveness in improving production and health outcomes
4. Increased ketone availability can have positive effects on metabolic health via the attenuation of inflammation and the improvement of insulin sensitivity.

Rico & Barrientos-Blanco 2024

Discussion

1. True. Associations depend on which outcome, when BHB is measured, how high, glycemia, and level of milk yield.
2. True. Still searching for what triggers inflammation before calving. Doesn't refute the potential utility of HYK as a pragmatic marker.
3. ± true. Doesn't refute the potential utility of HYK as a pragmatic marker or that treatment with glycol reduces DA and culling risk.
4. Based largely on studies in non-lactating humans and lab animals. Experimental models in mid- or late-lactation or dry cows ≠ relevant for the complex milieu of transition cows

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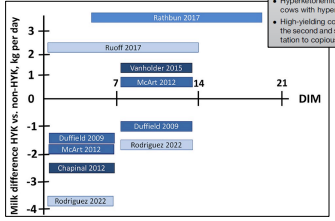
Hyperketonemia VCNA 2023

A Marker of Disease, a Sign of a High-Producing Dairy Cow, or Both?

Sabine Mann, Dr. med. vet, PhD, Dip. ACVPM, Dip. ECBHM (Epidemiology), Jessica A.A. McArt, DVM, PhD, DABVP (Dairy Practice)*

KEY POINTS

- Focus hyperketonemia diagnosis at 3 to 9 days in milk.
- On-farm blood β-hydroxybutyrate (BHB) meters offer the best balance of testing accuracy, convenience, and economics when used appropriately.
- Oral propylene glycol administered for 3 to 5 days remains the most evidence-based treatment of hyperketonemia.
- Hyperketonemic cows with concurrent hypoglycemia benefit more with treatment than cows with hyperketonemia alone.
- High-yielding cows will often have blood BHB greater than or equal to 1.2 mmol/L during the second and subsequent weeks of lactation, which is indicative of an appropriate adaptation to copious milk production.



The association of HYK with milk yield varies among studies.

It appears to be conditional on:

- The timing of onset (week 1 >> worse than week 2)
- Duration
- Threshold (BHB > 1.2 to 2.0 mmol/L)
- Glycemia
- … and probably other uncharacterized variables

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Would experimental challenge studies be better evidence?

- Classic ketosis induction model (Drackley et al 1991, 1992)
  - ~ 20% feed restriction from 14 to 42 DIM ± fed butanediol at 7% of diet DM
  - Did not produce HYK (plasma BHB < 1.0 mmol/L)
  - Reduced DMI and milk yield
- Comprehensive critique in Rico and Barrientos-Blanco (2024)
  - No HYK induction models recapitulate the milieu of HYK in transition cows

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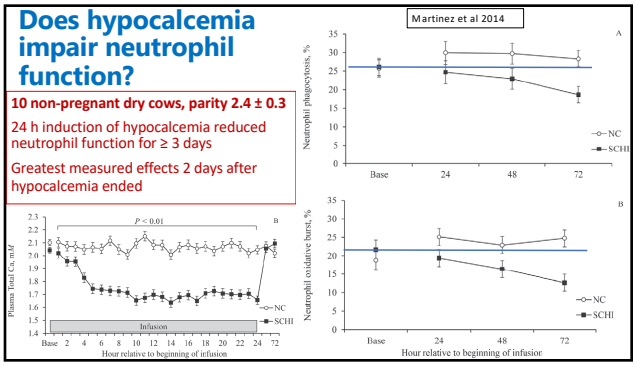
Does hypocalcemia impair neutrophil function?

10 non-pregnant dry cows, parity 2.4 ± 0.3

24 h induction of hypocalcemia reduced neutrophil function for ≥ 3 days

Greatest measured effects 2 days after hypocalcemia ended



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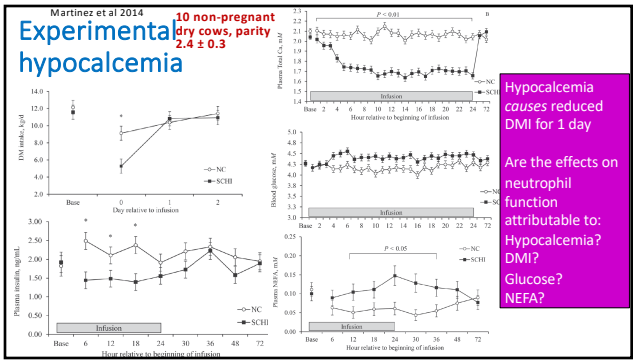
Experimental hypocalcemia

Martinez et al 2014

10 non-pregnant dry cows, parity 2.4 ± 0.3



Hypocalcemia *causes* reduced DMI for 1 day

Are the effects on neutrophil function attributable to:
Hypocalcemia?
DMI?
Glucose?
NEFA?

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If ketosis were a problem, treating it would result in more milk

- A condition can be problematic whether or not therapy is efficacious
  - See: mastitis
- Milk isn't the only outcome that matters
  - Cows culled with DA and/or low production aren't in the calculation of
    - Week 4 milk
    - Test day milk
    - Peak milk
    - 305 milk
- Treatment of HYK (and most other diseases/disorders) doesn't entirely mitigate associated increases in subsequent disease, production, or fertility
  - More selective treatment (e.g., concurrent HYK and low glucose) and/or novel approaches (e.g., addition of anti-inflammatory therapy) are promising

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Is it useful?

- Is it useful to track HYK trends in my herd?
- Is there a benefit to identifying and treating cows with HYK?
- The utility of monitoring and treating HYK is likely conditional:
  - Herd-specific baseline risks
    - DA
    - Early culling
    - Pregnancy at 1st AI and/or 21-day pregnancy rate
  - Cow-level
    - Milk yield
    - Blood glucose
  - Week 1 > week 2

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Conclusions

- Establishing mechanisms and causality is interesting and helps advance science
- It's not necessary to find useful actions
- Relevant experimental models for transition dairy cows are difficult to establish
- Well designed, sufficiently large randomized controlled trials are often the best evidence to support dairy management decisions
- Observational studies are usually necessary to assess the effects of health disorders. Properly understood, they also advance science.

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