NUTRITION AND THE MICROBIOME AND THEIR ROLE IN CHRONIC ILLNESS

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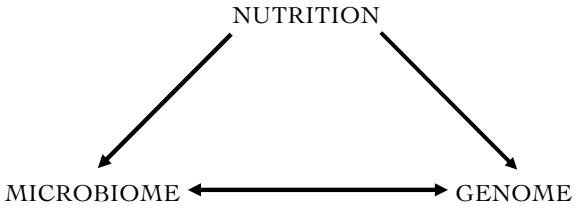

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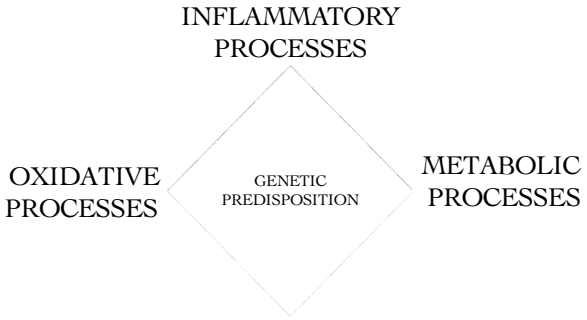

Diseases arise because of genetic predisposition to one or more of these stressors

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The Basic 6 Steps

- STEP 1 - **Species Appropriate Diet - Optimised Nutrition**
- STEP 2 - **Avoidance of Inflammatory Foods**
- STEP 3 - **Reduction of Cellular Oxidative Stress**
- STEP 4 - **Maintaining a balanced Gut Microbiome**
- STEP 5 - **Nutrigenomics - Individualised Nutrition based upon Genetic Predisposition**
- STEP 6 - **Psychological Wellbeing**

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STEP 1: Biologically Appropriate for the Species

- **Evolution of the species**
  - Dentition
  - Gastrointestinal tract development
  - Enzymes
- How much has each companion animal species changed over their evolutionary period adapting to living with man

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Omnivore vs. Carnivore?

Stomach PH = 1
STRONG ACID

5-8 hrs transit
5 types of teeth
None of which
GRIND

The Amylase Debate!

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- **Best supports a healthy bacterial flora (gut microbiome) - species specific**
  - Germ-free mice and mice treated with a heavy dose of antibiotics responded poorly to a variety of cancer therapies typically effective in rodents
  - The commensal microbiota affect inflammation and, through that or through other mechanisms, affect the development of chronic diseases including carcinogenesis

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Step 2: Avoidance of Inflammatory Foods

- Dairy
- Excess saturated fats - fatty meat
- Plant oils e.g. sunflower - AA is converted to inflammatory compounds
- Grains - wheat, corn, rice, barley - sugars - fluctuate blood sugar levels
- Solanaceae: Potato, tomato, pepper, aubergine - **LECTINS**

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Dietary Management to Balance Lectin Content

Can become lectin-sensitive because of:

•**Genetic predisposition**

•Failure or lowered genetic or environmentally induced protection of gut mucosa by **secretory IgA**

•Certain **bacteria** and **viruses** damage cells making them susceptible to lectin antibody/antigen reactions

•**NSAIDs** (non-steroidal anti-inflammatory drugs) or **other drugs**, which increase gut permeability, allow lectins to enter the general circulation.

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- E Numbers, colourants, nitrites, trans fats and preservatives
- Heavy metals - Tuna
- Aflatoxins

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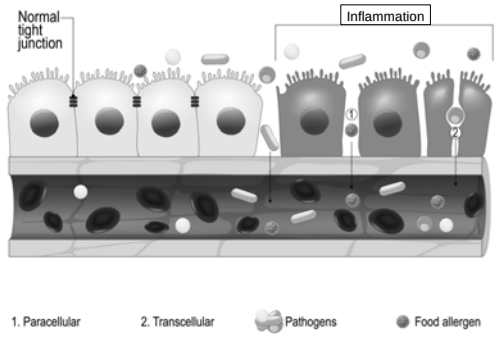
LEAKY GUT



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Early diagnosis of leaky gut in dogs

- Newer tests use saliva – e.g. Nutriscan, or faeces
- Measures IgA or IgM antibodies to foods in saliva
- Antibodies to foods appear in saliva before GI tract clinical/biopsy diagnosis of IBD or "leaky gut syndrome"
- Saliva testing can thus reveal the latent or pre-clinical form of food sensitivity

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Step 3: Reducing Cellular Oxidative Stress

Involves **genetic regulation & gene transcription** in health & disease

•Elevation of intracellular **free radicals (Reactive Oxygen Species = ROS)**

•Elevated ROS levels damage cells and tissues, causing disease

•Produced in large amounts during infection and disease

•ROS exist even in normal health

•Excess oxidative stress causes **mitochondrial dysfunction**

(Mandelker, JAHVMA, 41:22-24, Winter, 2016)

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Cellular Oxidative Stress & Chronic Disease

•Oxygen-derived free radicals cause cell toxicity

•Even in healthy state ~ 25% of oxygen forms free radicals

•In unhealthy states **up to 75% of oxygen becomes free radicals**

•**Common causes** : toxaemia, infections, hypoxic-ischaemia, hyperglycaemia, xenobiotics (drug metabolism), hyperlipidaemias, hyperproteinaemias, cancers, phagocytic and immune reactions, and high metabolic rates

•Ageing tissues under oxidative stress

(Mandelker, JAHVMA, 41:22-24, Winter, 2016)

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Reducing cellular oxidative stress

- Calorie restriction
- Exercise
- Antioxidants - Food sourced vs. Supplements
  - Impoverished soils
  - CoQ10
- Reduction in drugs

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STEP 4: Maintaining the Gut Microbiota

- Microbes and animals working together for millions of years
- Trillions of dynamic organisms
- Comprised of bacteria, archaea, protozoans, yeasts, fungi, viruses and bacteriophages (at least!)
- When we eat we are feeding both them and us
- If we look after them they look after us
- **Commensal organisms** - Protecting us from pathogenic organisms - dynamic process
  - The gut and its microflora are the largest component of the bodies immune system

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- Metabolic capacity to produce and regulate compounds that reach the circulation and act to influence the function of distal organs and systems - **virtual endocrine organ**
- The gut microbiota has the capacity to produce 100's of bioactive molecules - many we cannot get from food
- Biochemical complexity exceeds that of the brain
- The gut microbiome weighs approx. **1-2 Kg** in the average adult human
- Culture independent techniques e.g. genomic approaches, PCR

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Large Scale Metagenomic Studies

National Institutes of Health-funded Human Microbiome Project
http://commonfund.nih.gov/hmp

European-funded Metagenomics of the Human Intestinal Tract consortium
http://www.metahit.eu

International Human Microbiome Consortium
http://www.human-microbiome.org

Enormous human and financial resource being poured into this research

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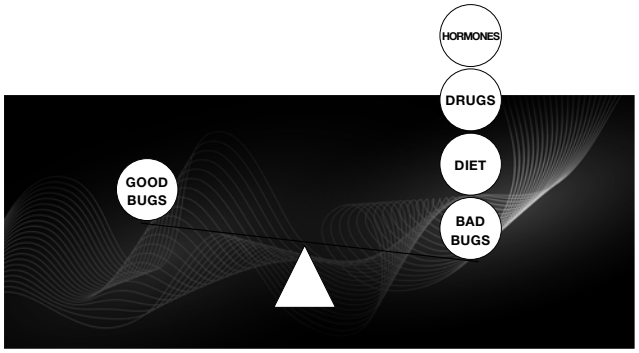

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The Canine Microbiome

- Results of the analysis of the canine fecal microbiome indicated a predominance of the phyla Firmicutes (24–40%), Bacteroidetes (32–54%), Fusobacteria (15–25%), Proteobacteria (5–6%) and Actinobacteria (0.3– 1.4%)
- Analysis of the canine GI and fecal microbiota composition, its function, production of metabolites and immunological properties is far from complete, even though data on the microbiome are accumulating

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Changes in the Canine Microbiome in Disease

- **Acute diarrhoea:**
  - Increased abundance of Clostridium spp., E.Coli, Lactobacillus and Enterococcus spp.
  - Reduction in Faecalibacterium, Runminocccaceae and Blautia spp.
- **Chronic diarrhoea:**
  - Increased abundance of Bacteroides spp. Bifidobacterium spp., Lactobacillus spp. And E.Coli
  - Reduction in Fusobacteria, Ruminococcacaceae, Blautia spp. And Faecalibacterium spp.
- **IBD study:**
  - Increase in Gammaproteobacteria e.g. E.Coli
  - Decrease in Erysipelotrichia, Clostridia and Bacteroidia

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Are these changes a cause or a result of the aberrant immune reactions seen in the host?

It is now suspected that the bacterial changes are associated with **altered metabolic functions of the microbiota** e.g. decrease in SCFA concentrations, altered amino acid metabolism, changes in redox equilibrium, altered bile acid metabolism, leading to an exacerbation of the inflammatory state of the host

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Not just any old pre-, pro- or synbiotic!

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Definition of Pre-, Pro- and Synbiotics

- **Prebiotics**
  - Selectively fermented ingredients that result in specific changes in the composition and/or activity of GI microbiota, thus also being a benefit to the host organism (WHO 2002)
- **Probiotics**
  - Live microorganisms, which when consumed in adequate amounts confer a health benefit to the host (Gibson & Roberfroid et al 2010)
- **Synbiotics**
  - Preparations combining prebiotic and probiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria, thus improving host welfare (Gibson & Roberfroid 1995)

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Mechanisms of action of Probiotics

- **Displacement of intestinal pathogens**
  - Interfere with their adherence to the intestinal mucosa
  - Induce mucous/mucin production
- **Production of antimicrobial substances**
  - E.g. Lactic acid, fatty acids, acetic acid
- **Enhancements of immune responses**
  - Maintenance and fortification of tight junctions
  - Induction of IgA and Beta-defensin production
  - Prolonging the survival of IEC's
- **Up regulation of various metabolites**

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Organisms used in Canine Products

- In Europe (2016) 4 bacterial strains/products had been examined by the **European Food Safety Authority (EFSA)**
  - Enterococcus faecium - 2 different strains
  - Lactobacillus acidophilus
  - Bifidobacterium spp. animalis
- Other studies have looked at:
  - Sacchoromyces boulardii (yeast sp.) in **IBD** and **protein losing enteropathy** (Bresciani et al 2014)
  - Lactobacilli (4 strains: acidophilus, plantarum, paracasei and delbrueckii spp.), Bifidobacteria (3 strains: breve, longum, infantis) and Streptococcus thermopiles in **IBD** (Rossi et al 2014)

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Research Project - Adored Beast Apothecary Canada (Lee.J 2017)

- **Research Project**: Isolation and characterisation of Lactobacilli for use as a host specific canine probiotic
- The probiotic candidates were subjected to a series of tests including tolerance to simulated gastric and intestinal conditions, production of antibacterial substances, host immune modulation capabilities (using two canine cell lines), antibiotic resistance testing, and strain stability.
- Isolated 2 lactobacillus sp.:
  - **L.casei**
  - **L.fermentum**

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RESEARCH STUDY

Production of inhibitory metabolites

	C. perfringens 8533	*C. perfringens* 15	*S. enterica* sv. Typhimurium	*E. coli* E2348/69
K9-1	YES	YES	YES	YES
K9-2	YES	YES	YES	YES

Many lactic acid bacteria produce metabolites that are inhibitory toward other bacteria. Using the deferred inhibition assay, we tested whether the canine isolates had inhibitory activity toward common canine gastrointestinal pathogens. We determined that both K9-1 and K9-2 produce substances that inhibit the growth of C. perfringens - CLOSTRIDIUM, S. Typhimurium - SALMONELLA, and enteropathogenic E. COLI.

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RESEARCH STUDY

Antibiotic resistance profiles

	Gentamycin	Kanamycin	Streptomycin	Tetracycline	Erythromycin	Clindamycin	Chloramphenicol	Ampicillin
K9-1	YES	YES	YES	YES	YES	YES	YES	YES
K9-2	YES	YES	YES	YES	YES	YES	YES	YES

Due to growing concerns regarding antibiotic resistance and the transmission of antibiotic resistance genes, the minimum inhibitory concentration (MIC) for 8 clinically-relevant antibiotics was determined for K9-1 and K9-2. This is in accordance with the European Food Safety Authority (EFSA) regulations. Both K9-1 and K9-2 had MICs equal to or below the breakpoint values specified by the EFSA for all antibiotics tested.

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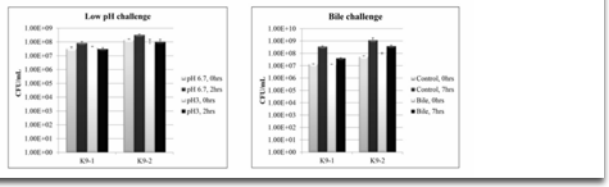
RESEARCH STUDY

Survival in simulated gastric and intestinal conditions

The ability to survive transit through the gastrointestinal (GI) tract is an important characteristic of direct-fed microbials. We challenged K9-1 and K9-2 in nutrient medium at acidic pH (to mimic the stomach) or containing bile (to mimic the intestine) for the indicated time intervals. Both K9-1 and K9-2 tolerate incubation in broth at pH 3 for 2 hours. K9-1 and K9-2 both grow in broth containing 5% ox bile. This suggests both isolates may survive transit through the canine GI tract. Additional research: A CONFIRMED REAL WORLD FEEDING TRIAL WAS CONDUCTED AND SUPPORTS THE SIMULATED STUDY.



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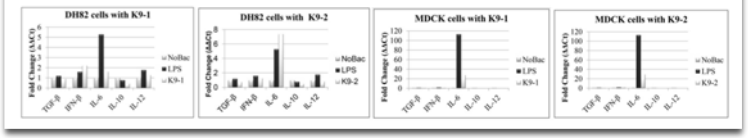
RESEARCH STUDY

Immune Modulation

DH82 (canine macrophage-like cell line) or MDCK (canine epithelial cell line) cells were co-incubated with K9-1 or K9-2. Cell lysates were analyzed for their cytokine expression levels by qRT-PCR.

Supports the body's response to increase the immune system to fight example harmful pathogens or decrease the immune response in autoimmune situations



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How can we find out about the health of an individuals gut flora and gut health?

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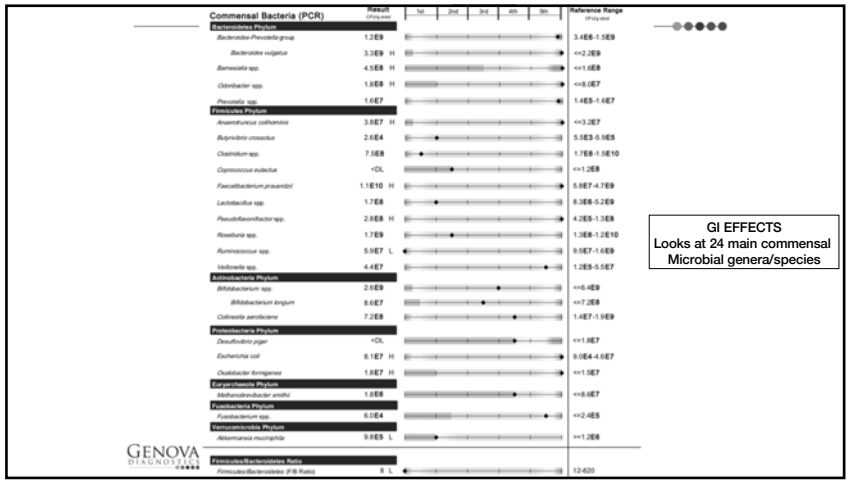

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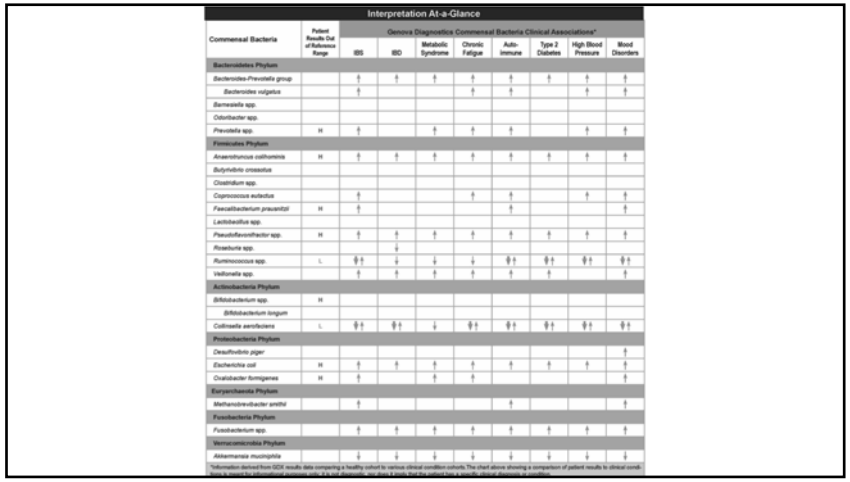

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You can also look for:

- The **metabolic indicators** that demonstrate specific and vital metabolites produced by bacteria e.g. short chain fatty acids (nutrients for colonocytes) and beta-glucaronidase
- Measure of e.g. how well your food is being digested, how inflamed the gut is etc.
- **Pathogenic bacteria and parasites**
- **We are on the very first step in functional medicine in dogs and not near the staircase in cats!**

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Step 5: Individuliased - The Future Gold Standard

- Nutrition should not simply be species appropriate but age and individually appropriate
- **Nutrigenomics** - the study of the mechanisms by which nutrients and dietary bioactive molecules affect gene expression
- Nutrition modulates gene expression through the genome and metabolism plus epigenetic factors (gene programming)

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An example of nutrigenomics in action:

- The Speed Gene test (PlusVital) examines the **Myostatin gene** that is responsible for muscle development and muscle fibre type
- The test categorises horses into three distinctive types:
  - **C:C** Sprint/Mile Types
  - **C:T** Middle Distance Types
  - **T:T** Staying Types
- Thoroughbred horses that are **T:T (***suited to exercise requiring stamina*) genetic types, produced significantly lower cellular levels of **CoQ10** than the other (C:C and C:T) genetic types, but that these levels can be restored with supplementation.
- In field trials, it was demonstrated that CoQ10 concentration in the muscle increased by 40% following nine weeks of oral supplementation.

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- cDNA microarray technique's make it possible to understand many of the factors controlling the regulation of gene transcription
- Now being used to evaluate the effects of nutrient management schemes on gene expression
- In the future it may be possible to identify for an individual the nutrients needed to optimise the their gene expression for optimal health

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Step 6. Psychological Wellbeing

- Understanding the needs of the individual
  - How they express stress
- The 'chicken and egg' in the effects of stress on the microbiome
- Learning to accept emotional expression in animals

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CANCER CONSIDERATIONS

Cancer is both a genetic and a metabolic disease

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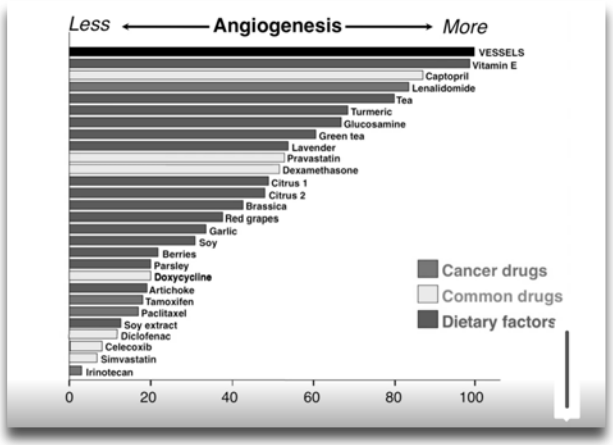

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Anti angiogenic foods - humans

Green tea	Red grapes	Lavender
Strawberries	Red wine	Pumpkin
Blackberries	Bok choy	Sea Cucumber
Raspberries	Kale	Tuna
Blueberries	Soy beans	Parsley
Oranges	Ginseng	Garlic
Grapefruit	Maitake mushroom	Tomato
Lemons	Licorice	Olive oil
Apples	Turmeric	Grape seed oil
Pineapple	Nutmeg	Dark chocolate
Cherries	Artichokes	Others

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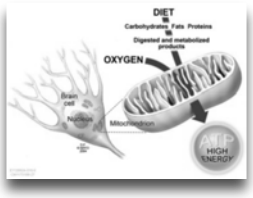
- The **Warburg Effect**
  - **Aerobic Glycolysis**
    - = increased glucose uptake and the production of lactic acid in the presence of oxygen
- **Mitochondria** - dysfunction - impaired cell respiration



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Chronic Calorie Restriction

- Numerous studies show that dietary energy restriction is a general metabolic therapy that naturally lowers circulating glucose levels and significantly reduces growth and progression of numerous tumour types to include cancers of the mammary, brain, colon, pancreas, lung, and prostate in humans
- Fats and especially ketone bodies can replace glucose as a primary metabolic fuel under calorie restriction

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Brain Cancer - An exception

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The Importance of Diet

- The importance of diet in the treatment and prevention of cancer is long established
- Max Gerson
- Dr Grimmer – the pure vegetarian diet
- Budwig Diet – Flax seed oil/cottage cheese 1:2
  - Chia Seed Oil does not need to be converted to Omega 3 therefore may be better in dogs
- As cancer progresses, alterations in the normal patient metabolism occur and often these changes are not reversible without treatment and adequate nutritional support, even after surgery when it is applicable.

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Difficulties in advanced cases

- Inappetence
- Physical difficulties e.g. Ulcerated mouth tumours
- Swallowing difficulties
- High energy requirement OR not?
- Vomiting/nausea
  - Primary through e.g. Stomach ulceration
  - Generalised toxaemia
- Changes in hormone cascades e.g. Insulin and glucose metabolism

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Acid/Alkali - Na/K

- Chronic disease and the acid environment - cancer proliferates rapidly in an **acid** environment
- Cancer establishes its own **local acid environment** that is no longer dependent upon the blood pH
- Reduction in acidifying foods and emphasis on **alkalinising foods** e.g. Alfalfa juice, leafy vegetables
- Agents known or believed to be carcinogenic **decrease** the concentration of **potassium** and **increase** the concentration of **sodium** in the cells
- **Anti-carcinogenic agents** have the opposite effect i.e. they **increase the intra cellular potassium levels** and lower the sodium levels.

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The Cancer Cell

- Tumours convert carbohydrate (glucose) easily into energy
- Tumours exist within a 'wound healing' environment – energy hungry
- Many but not all neoplastic cells **do not possess the metabolic machinery required to oxidise fats and ketones**

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Basic Requirements

- The diet must favour potassium uptake in the cells
- The diet must principally provide energy in the form of fats and protein
- The diet must provide adequate protein for repair
- The diet must provide the vitamin and mineral spectrum necessary for:
  - The enhanced production of key immune modulators and enzymes involved in the breakdown of tumour products.
  - To support **mitochondria**
- These vitamins and minerals must be in a usable absorbable (bio-available) form for the body
- The diet must not add to the toxic load on the liver and kidneys

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Raw vs. Commercial

- In an ideal world – RAW

  BUT
- We must not underestimate the strain on the system of converting to raw in an **immunocompromised** animal that has been fed on commercial diet all its life
- Potential detox crisis
- Beware whole bones – assess the metabolism
- Owner ability – bad raw can be as bad if not worse than the best commercial
- Now good formulated raw diets in the UK
- Pre,Pro and Syn -biotics
- Digestive enzymes

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Processed

- There are now possibilities if processed kibble is the only option:
  - **Commercial Cancer Diet**
    - Added Arginine, n-3 oils etc.
    - Shift from carbohydrate loaded diet
    - Emphasis on high fat and protein
  - Commercial diets emulating raw in the split of nutrients and mostly grain free – BIOLOGICALLY APPROPRIATE FEEDS - 80:20 or 70:30
    - e.g. 70% protein and high in vegetable/fruit content e.g. Orijen, Ziwi Peak etc.
    - Liver and kidney function needs careful assessment if switching to these foods

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Home Produced Raw

- Meat and bone based - debate over vegetable BUT not all nutrients come from meat
- Emphasis on high quality grass fed meat (and vegetables?!)
  - Preferably organic BUT still scrub root vegetables and rinse fruits
- Eggs, cottage cheese (especially if the liver is affected), spirulina, wheat grass, alfalfa juice and barley greens.
- White meats and un-hung game if liver under severe pressure
- Offal e.g. Kidney/liver/heart in anaemia – human grade
- Digestive enzymes particularly if converting from processed/cooked food
- Probiotics

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Fats

- Neoplastic cells cannot oxidise fats
- Rations high in fat have been shown to normalise carbohydrate metabolism
- Prolonged survival times in study on high fat diets in canine lymphoma
- Fats also provide more calories per gram than protein and carbohydrates – poor appetite
- Biochemical response to food deprivation leads to substantial dependence on fat-derived fuels – animals that have not eaten for 24 hours
- Care to avoid liver overload – small meals little and often
- Particular care in cats
- Pancreatitis/cholangiohepatitis potential

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n-3 Fatty Acids

- Quality – poor fish oils contain high levels of carcinogens!!!!
- n-3:n-6
- Increasing the ratio reduces the number of pro inflammatory cytokines
- Longer disease free interval and survival time for dogs with Stage III lymphoma fed the experimental diet high in n-3 fatty acids
- Chia seeds rich source that does not need converting in the body

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Note of caution

- Reduction in platelet aggregation particularly noted in cats
- Potentially **unsuitable in tumours with a haemorrhagic tendency** e.g. Haemangiosarcoma
- Depressed immune function which may be due to increased tissue lipid peroxidation

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