Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis

Robert Kleemann, Lars Verschuren, Marjan J van Erk, et al. *Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis* Genome Biol. 2007; 8(9): R200.

Main Focus:

Animal models are being used in order to asses liver inflammation leading to atherosclerosis by measuring amounts cholesterol and its effect on the liver. Results difficult to be obtained by human subjects are found through the use of mice showing a possible correlation between dietary cholesterol intake and atherosclerosis. Information obtained in this way, may give more direct insight on how to prevent or reduce atherosclerosis based on the use of the animal model and compilation of knowledge through systems biology and metabolomics.

New Terms

Transcriptomics - classification and analysis of RNA molecules with coded genetic information transcripts and their formation, structure, and function in an individual.

Standard Gene Ontology (GO) – a project developed to give a common language to a gene products biology. This allows a same vocabulary for genes from different species to be compared based on their GO annotations. There is a set of terms for each category of molecular function, biological process, and cellular components.

HMG CoA reductase – The enzyme involved in the metabolic pathway that controls cholesterol and other isoprenoid production.

PDGF (platlet derived growth factor)- a protein that helps regulate cell division and growth especially in blood vessel formation, forming new blood vessels from previously existing blood vessel tissue.

CRP (C reactive protein) – a protein produced by liver and fat cells in response to immflammation, currently thought to help induce phagocytosis of damaged cells.

SAA (serum amyloid A)- a protein of the apolipoprotein family, found in vertebrates and inveretbrase produced by the liver associated with HDL in plasma.

ALAT(alanine amino transferase)- enzyme mostly found in the liver that catalyzes two parts of the alanine cycle.

ASAT(aspartate aminotransferase)- enzyme raised in liver damage, and converts aspartate to alpha-ketoglutarate to oxaloacetate and glutamate, and vice-versa.

RT-PCR – real time PCR used to produce DNA copies of an RNA template

SREBP1- Sterol regulatory element-binding protein 1, inhibition by sterols reduces the synthesis of more sterols through a negative feedback loop

Summary

Table 1

Effects of dietary cholesterol on plasma lipids and inflammation markers

	Con	LC	HC
Body weight (start) (g)	20.3 ± 1.5	20.8 ± 1.5	20.6 ± 0.9
Body weight gain (g)	0.4 ± 0.7	0.7 ± 0.8	0.6 ± 0.5
Food intake (g/day)	2.6 ± 0.2	2.9 ± 0.3*	2.5 ± 0.2†
Plasma cholesterol (mM)	5.9 ± 0.3	13.3 ± 1.9*	17.9 ± 2.4*†
Plasma triglyceride (mM)	1.7 ± 0.4	2.3 ± 0.3	2.1 ± 0.7
Plasma E-selectin (μg/ml)	44.3 ± 2.3	44.3 ± 6.3	55.1 ± 8.5*†
Plasma SAA (μg/ml)	2.8 ± 0.6	4.7 ± 1.7	8.3 ± 2.7*†
Plasma ALAT (U/mL)	48 ± 44	45 ± 22	75 ± 23
Plasma ASAT (U/mL)	260 ± 123	237 ± 57	569 ± 221*†

Three groups of female E3L mice were fed either a cholesterol-free (Con) diet or the same diet supplemented with 0.25% (LC) or 1.0% (HC) w/w cholesterol. Listed are the average body weight at the start (t = 0) of the experimental period together with the body weight gain, the average daily food intake and the average plasma levels of cholesterol, triglycerides, E-selectin, serum amyloid A (SAA), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). All data are mean ± standard deviation. *$P < 0.05$ versus Con; $^{\dagger}P < 0.05$ versus LC (ANOVA, least significant difference *post hoc* test).

*(http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2375038&rendertype=table&id=T)

The correlation between the effects of cholesterol production through the above mentioned article, using mice as animal models, shows how systems biology can offer a deep insight into many diseases. Based on the similarities of genes and proteins expressed across

animal populations, research can gain valuable knowledge into bringing together how a system works from all aspects including a molecular, genomic, and biological view.

The article relates specifically to the biochemistry metabolism course based on how the system works through positive and negative feedback, showing metabolic reactions and how they may be affected by particular changes of an addition, deletion, or change of a needed protein, enzyme, or other metabolite.

Atherosclerosis develops when LDL's or low-density lipoproteins are oxidized by mostly free radical oxygen molecules. Because of the amount of oxygen in blood, atherosclerosis develops mainly in arteries and not veins. Blood in veins is oxygen poor.LDL has a globular structure with a hollow center to carry cholesterol generally used to generate vitamin D or brain tissue. Usually artery damage caused by LDL becomes attacked by macrophages and T lymphocytes. These white blood cells cannot process the LDL however and usually swell and rupture depositing more cholesterol on the artery wall and repeating this cycle. A plaque then forms which causes the muscle cell to grow and form a cover over the affected areas causing a narrowing of the artery, reducing blood flow and increasing blood pressure.

CRP or C reactive protein is largely found in areas of inflammation, tissue damage, and infection. More doctors are currently using CRP levels to asses for cardiovascular disease. It is unclear whether CRP is a risk marker or factor of disease. In the mice CRP levels were measure using RT-PCR. Each set of micewas fed either a high cholesterol diet, low cholesterol diet, or cholesterol free diet. Upon examination of cross sections of the livers, heart, and arteries of these mice, it was shown that many more pathways were activated in the mice given high cholesterol diets. These pathways included those involved in inflammatory responses.

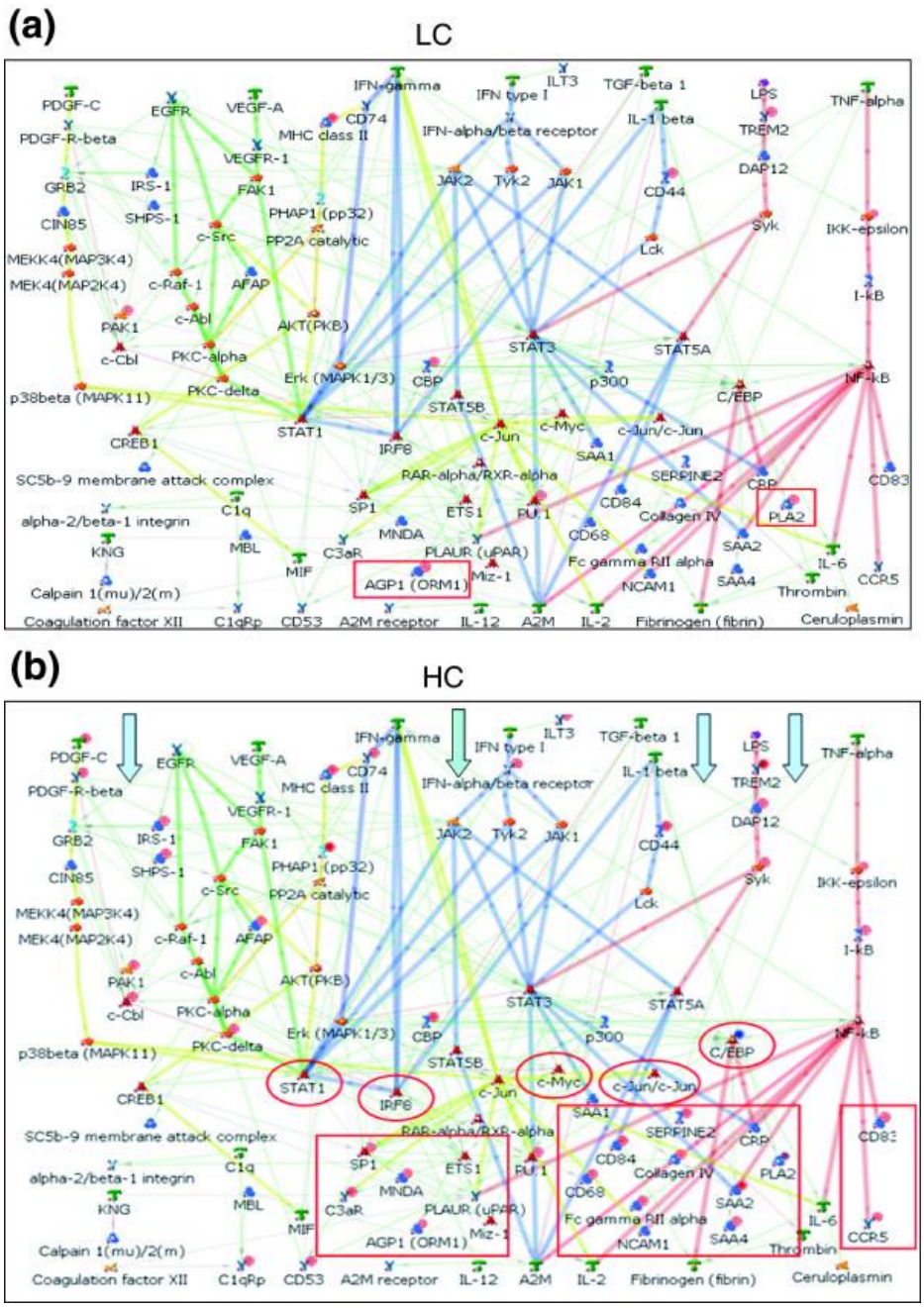

Figure 1

Analysis of the inflammatory pathways activated by the LC and HC diets. A master inflammatory network was generated in MetaCore™ by combining relevant inflammatory pathways. Differentially expressed genes in response to **(a)** LC and **(b)** HC treatment were mapped into this master network. The activation of the network by LC treatment was minimal, whereas HC treatment resulted in a profound activation of specific proinflammatory pathways (marked with blue arrows). Red circles indicate transcriptional node points and red rectangles highlight representative downstream target genes that were up-regulated. (http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2375038&rendertype=figure&id=F3)

This shows that the HC treatment turned on many genes whose products included correlation with inflammatory events involving proteases, complement, chemokines, heat shock proteins, adhesion molecules, integrins, acute phase proteins, and inflammatory transcription factors, leading the liver to an inflamed state not seen in the other Low Cholesterol treatment.

The conclusion of this research is that cholesterol and inflammation are linked by transcriptional regulators. The research done on these may give insight on new therapies. It is an excellent example of how systems biology brings together different divisions of science in seeing a clearer and well put together picture.

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Contribution of Research with Farm Animals to Protein Metabolism Concepts: A Historical Perspective

Werner G Bergen. *Contribution of Research with Farm Animals to Protein Metabolism Concepts: A Historical Perspective.* The Journal of Nutrition. Bethesda: Mar 2007. Vol. 137, Iss. 3; pg. 706, 5 pgs

Main Focus

Animals, particularly farm animals have been used for many years to trace the workings of proteins, carbohydrates, fats, and micronutrients. Lab animals were mostly used in research to follow protein metabolism, but scientists are also comparing these results with those of data obtained from farm animals. There is a great similarity between the animal models, both farm and traditional laboratory ones, that offer insight into human protein metabolism.

New Terms

IAAO – indicator amino acid oxidation, a method used to determine amino acid requirements for organisms.
(http://jn.nutrition.org/cgi/content/full/138/2/243)

DAAO – D-amino acid oxidase, a peroxisomal enzyme containing FAD spread from yeats to humans, to oxidize D-amino acids into imino acids producing ammonia and hydrogen peroxide
(http://en.wikipedia.org/wiki/D-amino_acid_oxidase)

PAA- plasma amino acids, a blood test done to determine problems with amino acid metabolism
(http://healthguide.howstuffworks.com/plasma-amino-acids-dictionary.htm)

Synergy – different parts work together to make a larger picture
(http://en.wikipedia.org/wiki/Synergy)

N-balance – nitrogen balance, difference of the dietary intake of nitrogen, usually proteins, and its excretion usually as urea and other waste products. Healthy adults excrete the same amount as is ingested, and are considered in N equilibrium.
(http://www.encyclopedia.com/doc/1O39-nitrogenbalance.html)

Nutigenomics – how genes and nutrition work together primarily looking at the effects of a nutrient on the genome, epigenome, proteome, and metabolome. (http://en.wikipedia.org/wiki/Nutrigenomics)

Nutriproteomics - the study of the nervous system and its protein complexes (http://en.wikipedia.org/wiki/Neuroproteomics)

Summary

By begin able to measure the amount of proteins of different classes of animals we can learn more about protein complexes and how they affect the body they inhabit. Tracing metabolites and recording results using methods such as IAAO, DAAO, PAA, and looking at the organisms N-balance, all provide an efficient and cheaper method for determining their significance. Following where these metabolites are located and seeing how they act in their metabolic pathways can give much insight on how to create new therapies or develop new pharmaceuticals for diseases. Using these tests we can research towards many protein associated diseases, especially those concerning diet. Depending on what an organism needs, changing its diet to correct a problem may be the easiest treatment to follow without adverse reactions or side effects. The animal models provide an excellent basis for this research. Traditional laboratory animal models can be compared to the farm animals easily and provide a larger scale, showing more relationships between the species. This provides more evidence towards human research and disease specifically involving metabolites and proteins.

TABLE 1 A timeline of protein metabolism and nutrition research issues and accomplishments during the 18th, 19th, 20th, and 21st centuries[1,2]

Century	Issues and accomplishments	Contributions by farm animals
18th	*Gaseous nitrogen not involved in animal N metabolism, N necessary in diet.	
19th	*Development and refinement of organic chemistry, early protein isolation and characterization, nitrogen balance concept and wide application. Emergence of theories of protein metabolism.	X
20th	*Single-ingredient diets, N or nutrient lack. Concept of supplementation by mixing feeds. Protein quality, identifying essential and nonessential amino acids	X
	*Protein and amino acid requirements; growth studies,	X
	factorial/N balance methods. Limiting amino acid concept.	X
	Amino acid imbalance; amino acid antagonism.	X
	*Dynamics of protein metabolism-turnover, use of isotopes	
	*Protein [energy] malnutrition; protein synthesis mechanism,	X
	Uterine and fetal nutrition	X
	*Application of plasma amino acids in assessment of protein status; indicator amino acids. In vivo tracer kinetics to study protein synthesis and degradation	X
	*Further refinement in protein/amino acid requirement studies as applied to humans; direct and indicator amino acid methods	
	*Amino acids as regulatory and signal transducer molecules.	
	*Pediatric protein metabolism; role of gut, muscle, and liver development	X
21st	*Pharmacology of excess amino acid intakes,	X
	Amino acid nutrigenomics; use of animal models	X

[1] Historical record of contribution specific to the issue by farm animals (left column, same line) is marked by X in the right column.
[2] This table is an overview, and space limitations prevented direct citations for each specific contribution by farm animals; however, many specific contributions are discussed in the text with appropriate citations.

(http://proquest.umi.com.ezproxy.rit.edu/pqdlink?vinst=PROD&fmt=4&filenumber=2&clientid=3589&ver=1&vname=PQD&RQT=309&did=1228435461&exp=05-02-2014&scaling=FULL&vtype=PQD&rqt=309&cfc=1)

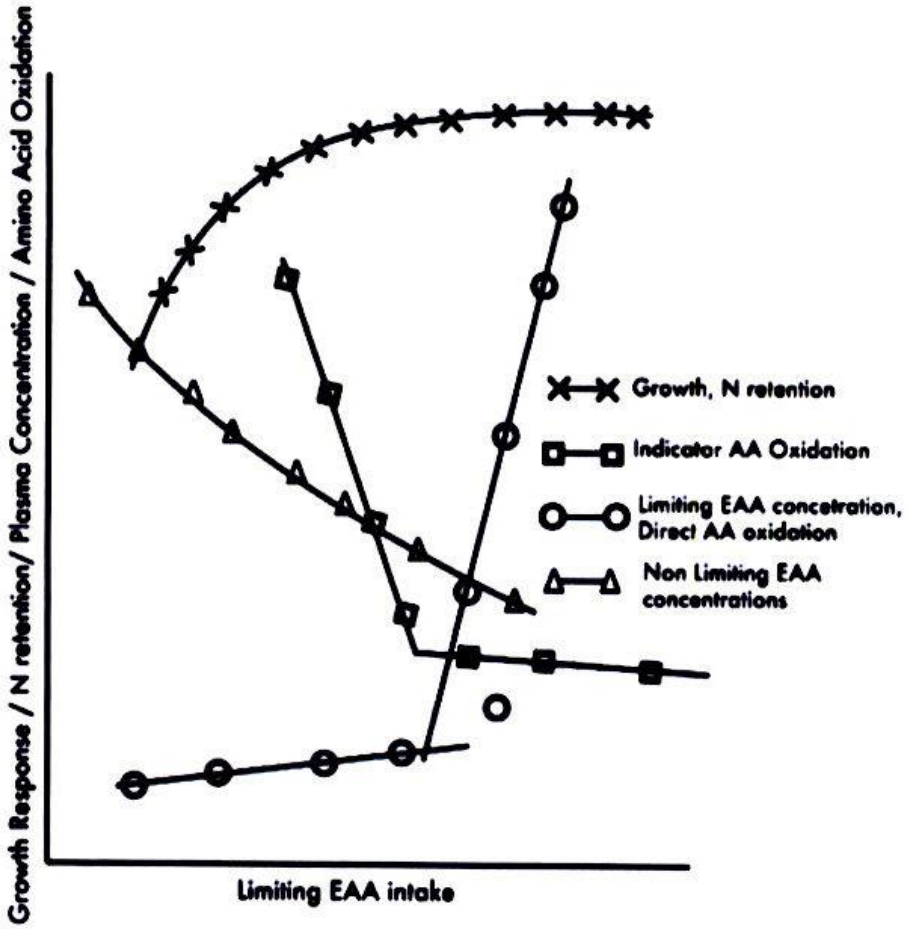

Figure 2 Effect of increasing limiting amino acid intake on plasma concentration of the limiting essential amino acid (*Lim EAA*), sum of plasma concentrations of nonlimiting EAA, growth response, direct and indicator amino acid oxidation, and N retention in an animal. Once the limiting amino acid requirement has been met under the given experimental conditions, plasma concentration and oxidation of Lim EAA rise rapidly, and the intersection between the 2 straight lines representing Lim EAA can be considered an estimate of the animal's requirement for that EAA (21,22,26,41). Nonlimiting EAA concentrations and indicator AA oxidation decline as Lim EAA intake increases to the estimated requirement.

(http://proquest.umi.com.ezproxy.rit.edu/pqdlink?Ver=1&Exp=05-02-2014&FMT=7&DID=1228435461&RQT=309&clientId=3589&cfc=1)

The above images show how animal models were used and what kind of testing was done on them.

This relates to the course because it is about metabolism. Using animal models to trace proteins and other macromolecules essential to life, we can create a larger picture in trying to help treat or prevent human disease. The similarities between human and animal models are starting to be understood in greater detail than ever before. Hopefully through the use of systems biology all disciplines can use each other's information and create highly specified maps of metabolic pathways. Once these pathways are figured out, it may mean an easier treatment for a specific patient or how to cure and treat a disease.

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