# APPLICATION OF ^{1}H NMR METABOLOMICS TO A MURINE MODEL OF INFLAMMATORY ARTHRITIS

Reza Dowlatabadi[1,2], B. Joan Miller[3], Frank R. Jirik[3], Hans J. Vogel[1] and Aalim M. Weljie[1,4]

[1]Metabolomics Research Centre, U. of Calgary, Canada; [2]Current Address: Dept. of Med. Chem., Faculty of Pharmacy, Tehran U. of Med. Sci., Iran; [3]Biochem. and Molecular Biology, U. of Calgary; [4]Chenomx Inc., Edmonton, Alberta, Canada. aweljie@ucalgary.ca
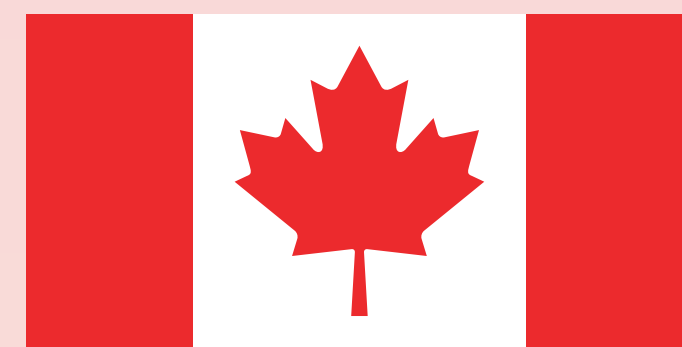
Introduction

Rheumatoid arthritis, a debilitating, systemic inflammatory joint disease impacting 1-2% of the population, may be accompanied by alterations in specific metabolites. As an initial approach to investigating this possibility in a well-defined system we selected a murine model of rheumatoid arthritis, the KBxN mouse. In this transgenic model, a systematic inflammatory response is generated towards the ubiquitously expressed glucose-6-phosphate isomerase enzyme. Here we examine the metabolite profiles of KBxN mice and contextualize them with respect to rheumatoid arthritis mechanisms (Figure 1).
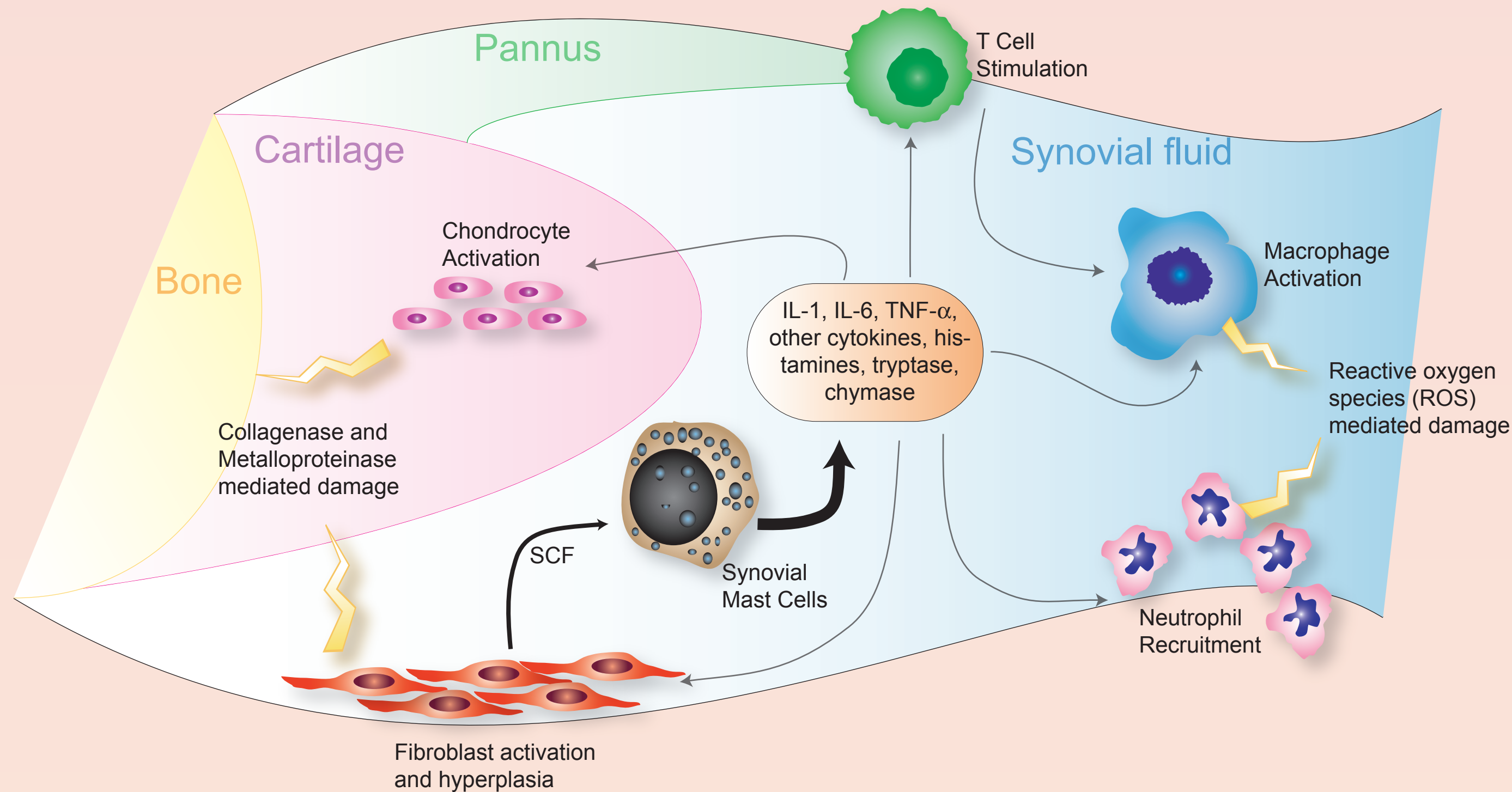

Figure 1: Schematic respresentation of selected mechanisms of rheumatoid arthritis. Stimulation of T cells occurs upon presentation of a self-antigen, which leads to macrophage activation. Cytokine release is implicated in many damage pathways. The autoimmune response leads to joint swelling and eventually degradation of the bone morphology.

Methods

Sera from arthritic populations of KBxN mice that are genetically-predisposed to arthritis (N=15), as well as healthy parent strain population (N=22) were analyzed using ultrafiltration followed by ^{1}H NMR spectroscopy.

A "Targeted Profiling" approach [1] was used to identify and quantify 54 metabolites using Chenomx NMR Suite (Chenomx Inc, Edmonton Alberta). Metabolite identification was confirmed using 2D methods and sample spiking.

Subsequent multivariate analysis was performed using SIMCA-P+ (Umetrics, Sweden) to build an orthogonal partial lease squares discriminant analysis (OPLS-DA) model. The model was verified using a cross validation approach, and a number of key metabolites identified as described below.
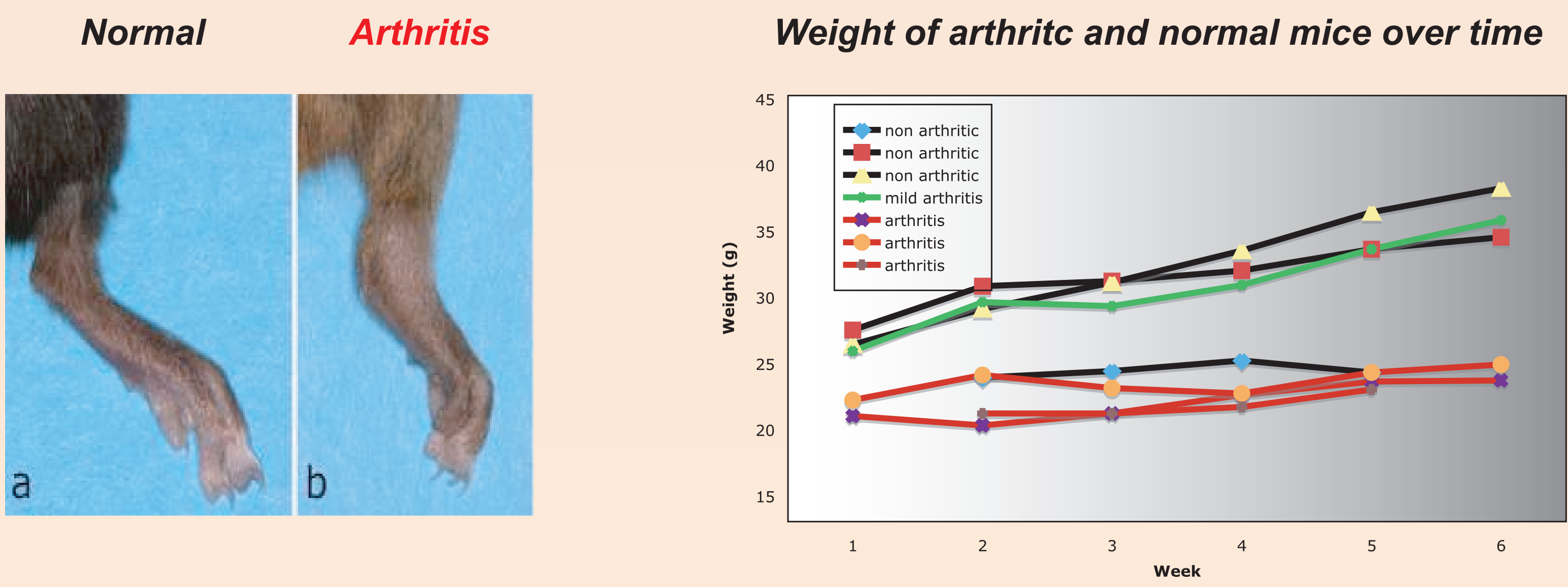
Results

Figure 2: Paw swelling evident in a) the genetic parent strain and b) the KBxN mouse model of arthritis.

Figure 3: Weight gain in the final six weeks of both arthritc and normal mice. In all cases, the arthritic mice gained less overall weight, and demonstrated a distinct plateau at < 25 g.

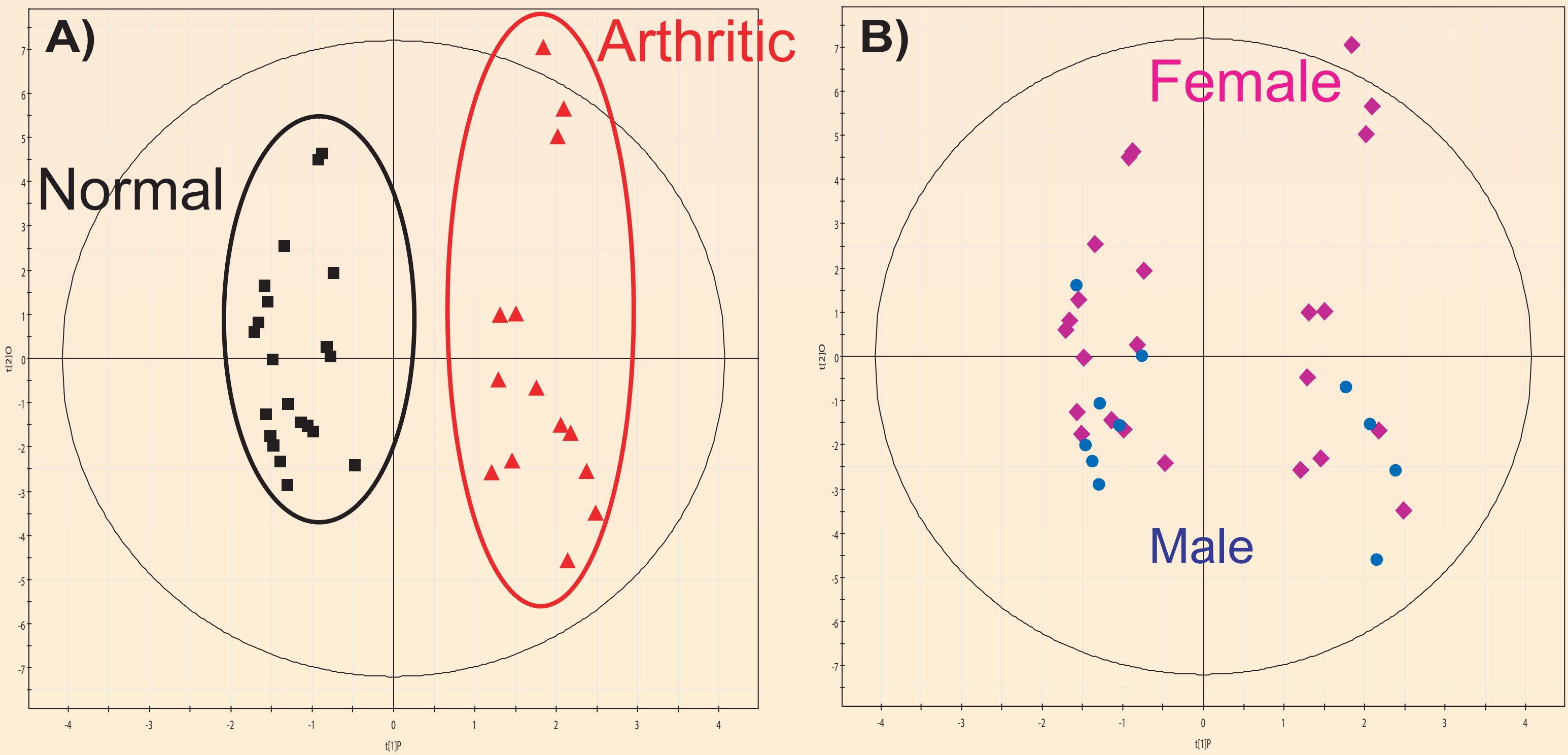*Scores plot from multivariate statistical modeling using OPLS*

Figure 4: Scores plot from the OPLS analysis of the metabolite concentrations obtained using the targeted profiling approach. A) Coloured according to type of mouse and B) coloured according to gender. Note that the first component in OPLS (x-axis) provides information on class separation, and as a result only the first component is considered for interpretation of metabolites relevant to arthritis. The first orthogonal component shows preliminary evidence of gender separation.

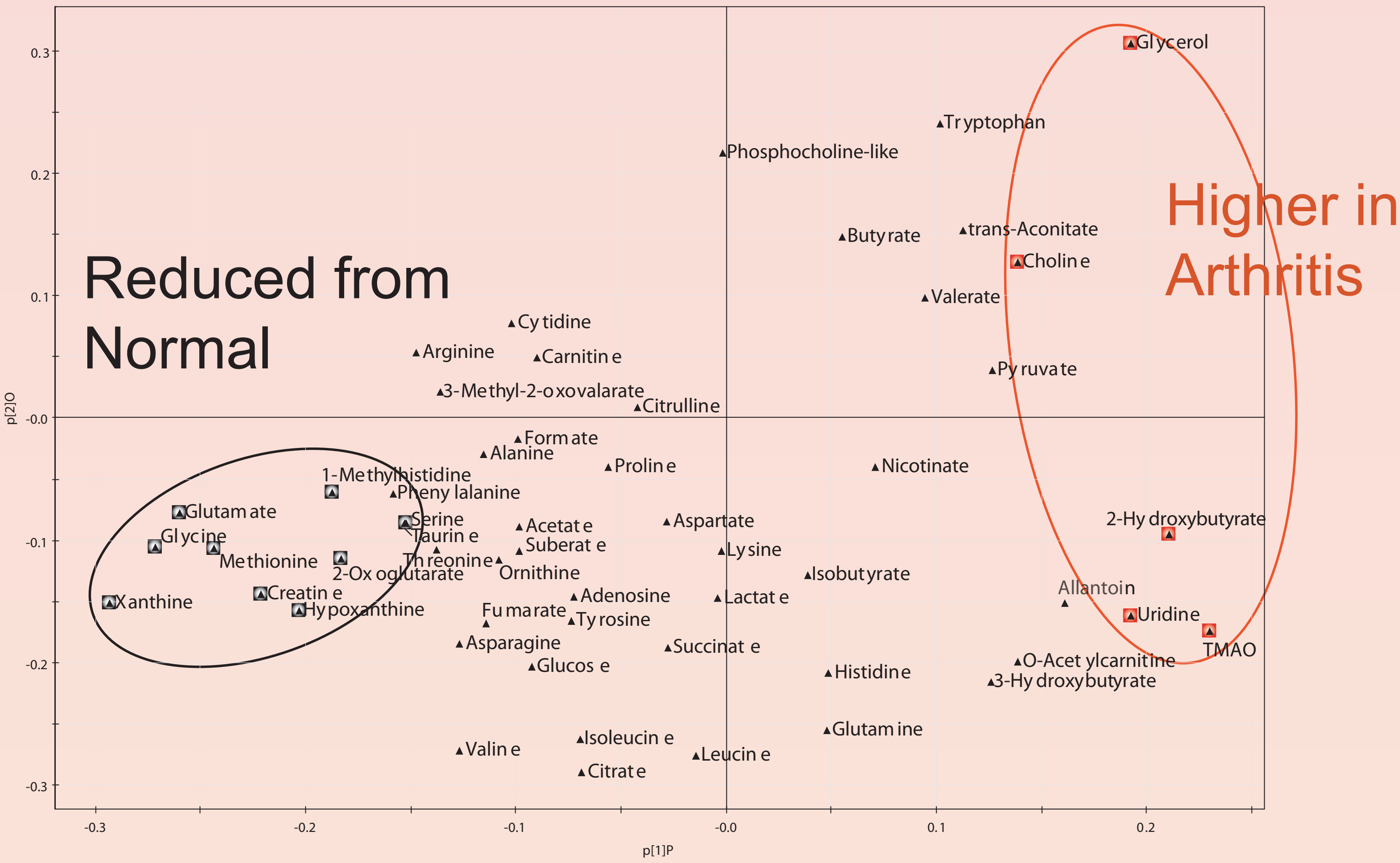*Loadings plot from multivariate statistical modeling using OPLS*

Figure 5: Loadings plot from the OPLS analysis of the metabolite concentrations obtained using the targeted profiling approach. Metabolites are highlighted either as reduced from the normal case (black) or elevated in the arthritis case (red). Metabolites were chosen if they had a score > 1 in the variable importance plot of the overall model.
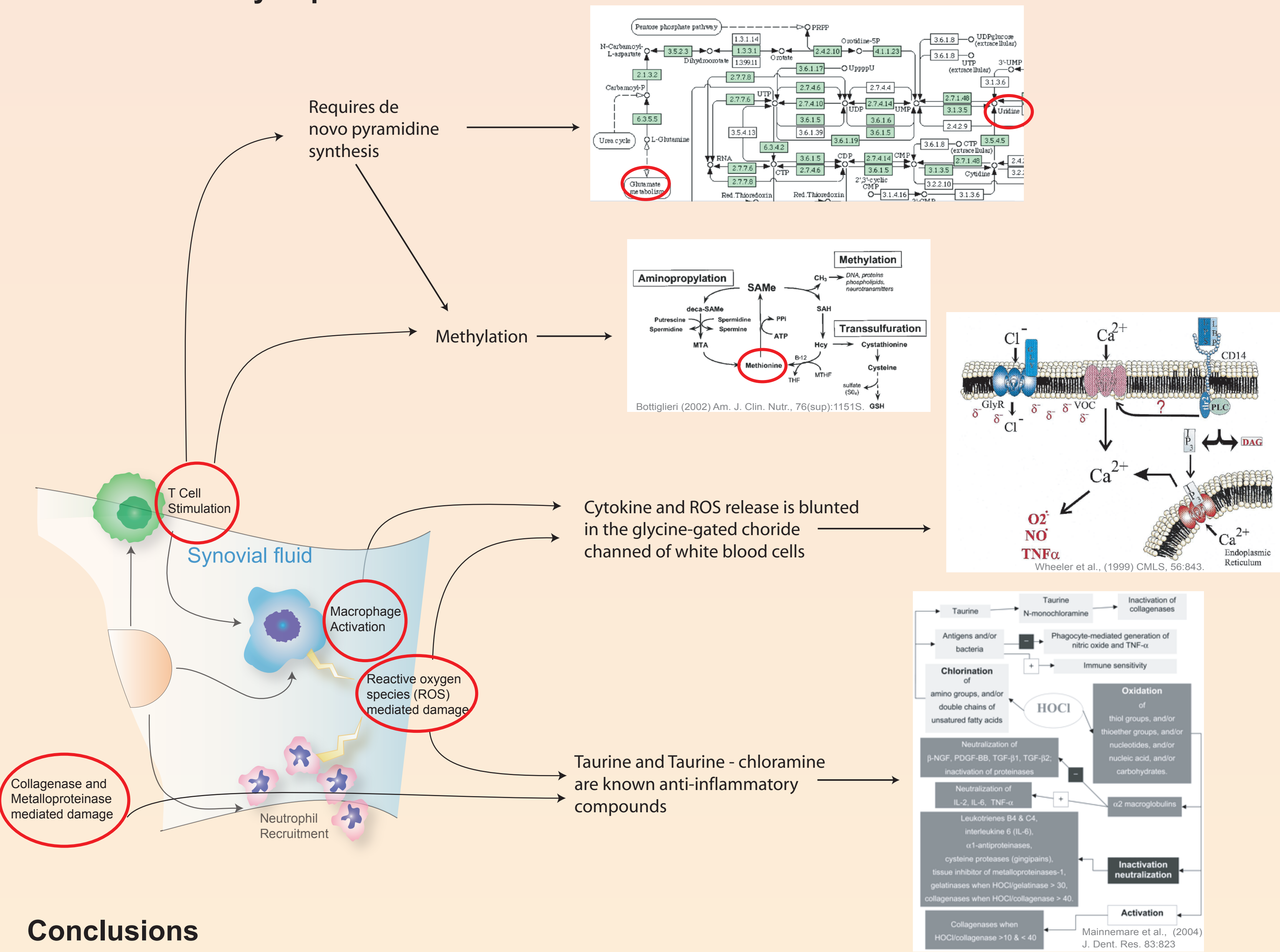
Summary of important metabolites

Decreased from Normal	Elevated in Arthritis
Xanthine	2-Hydroxybutyrate
Glycine	TMAO
Creatine	Uridine
Glutamate	Glycerol
Hypoxanthine	Choline
1-Methylhistidine	O-Acetylcarnitine
2-Oxoglutarate	
Methionine	
Taurine	
Threonine	

Potential physiological role of metabolites

Lipid metabolism
  – Glycerol, choline, acetylcarnitine, threonine
Nucleic acid metabolism
  – Xanthine, hypoxanthine, uridine
Reactive oxygen species (ROS) metabolism
  – Taurine, Glycine
Methylation
  – Methionine
Immune response in macrophages
  – Glycine (via glycine-gated chlorine channels)

Selected Pathway Implications

Requires de novo pyramidine synthesis

Methylation

Cytokine and ROS release is blunted in the glycine-gated choride channed of white blood cells

Taurine and Taurine - chloramine are known anti-inflammatory compounds

Conclusions

- Metabolite profiling of sera from mice models of human disease is a viable way to understand pathological mechanisms, and provide a means for evaluating the function of disease modifying drugs
- In the case of the KBxN mouse model, gender has little effect on an appropriately developed multivariate model.
- Some of the metabolic changes are likely related to dietary considerations (e.g. lipid metabolism), although a number of specific inflammatory biomarkers are also evident
- Our results attest not only to the complexity of systemic inflammatory responses, but also the power of the experimental approach in being able to reveal such a wide variety of biomarkers.

Acknowledgements