The Importance of Nutrition for Children with ASD

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Justification of ASD research

- "Milk link to brain disorders"

  (Cade *et al. Autism and schizophrenia: intestinal disorders;* Nutritional Neuroscience. 1999; 2: 57-72)

- Responses to dietary intervention

  (Knivsberg *et al. Reports on Dietary Intervention in Autistic Disorders;* Nutritional Neuroscience. 2001; 4: 25-37)

- Food fragments in urine: exorphins

  (Shattock *et al. Role of neuropeptides in autism and their relationship with classical neurotransmitters.* Brain Dysfunction. 1990; 3: 315-327)

- Responses to multimodal intervention

  (Cosford RE. Clinical data; Ellis EB. Clinical data; Fewtrell D. Clinical data; Mason L. Clinical data)

Evidence of immune system dysfunction

- High correlation between prevalence otitis media and autism (Kontstantareas M et al, Ear infections in autistic and normal children. *J Autism and Dev Dis* 17:585, 1987)
- Earlier age of otitis media correlates with more severe autism (ibid)
- Increased incidence otitis media correlated with more severe form of autism (ibid)
- T cell abnormalities previously noted in children with autism - ↓NK cells, ↓ CD 4 (Warren R.et al Immune abnormalities in patients with autism. *J Aut Develop Dis.1986;*16:189-197; Gupta et al. Dysregulated immune system in children with autism. *Autism Devel Dis*. 1996;26:439-452)

Why investigate autism?

- Incidence of autism was previously estimated to be 4 in 10 000 (1950's)
- UK study estimated incidence is 1 in 150 children (Chakrabarti S, Fombonne E, *JAMA* 2001;285(24):3093-3099)
- More recent UK study from Cambridge University found 157 in 10 000 children affected by an ASD (1 in 38 boys) (Baron-Cohen S, Scott FJ *et al*. Br J Psychiatry. 2009 Aug;195(2):182)
- Increased gut permeability in autism (D'Eufemia P et al. Abnormal intestinal permeability in children with autism. *Acta Paediatr* 1996:1076-9)
- Evidence of biochemical anomalies from preliminary data (Ellis EB *et al*. Biochemical profiles of children with autism: a preliminary study. Nutrition Society of Australia Inc. ASM Proceedings, 2000)

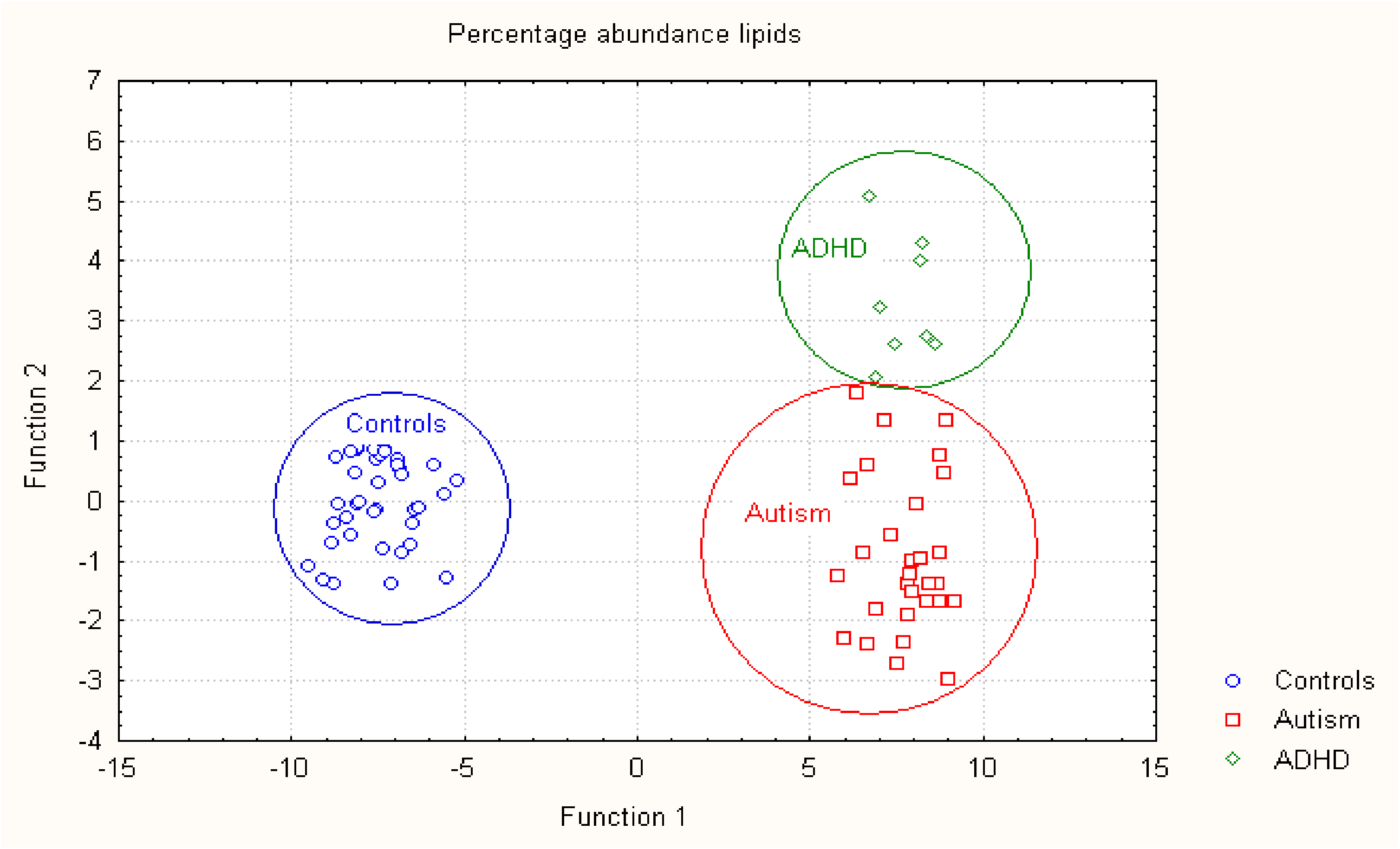
Gut Permeability

- A new study has found that increased gut permeability is present in both children with ASD (36.7%) as well as their relatives (21.2%)
- Compared with 4.8% of controls (children without ASD)
- Those on a GF/CF diet had significantly less intestinal permeability

De Magistris L *et al. J Pediatr Gastroenterol Nutr* 2010 Jul 28.

Biochemical anomalies

(Ellis EB *et al.* Biochemical profiles of children with autism: a preliminary study. Nutrition Society of Australia Inc. ASM Proceedings, 2000)



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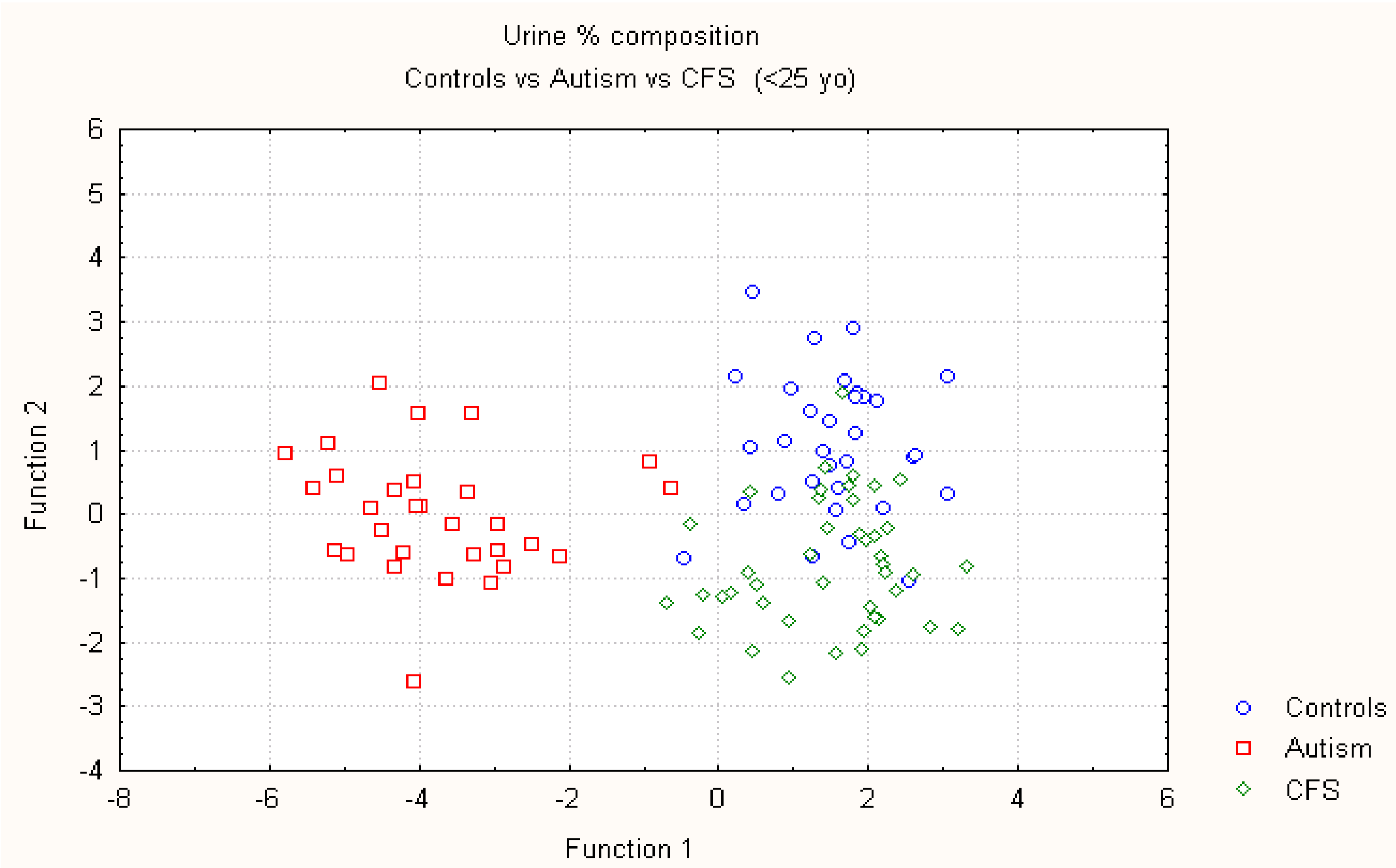

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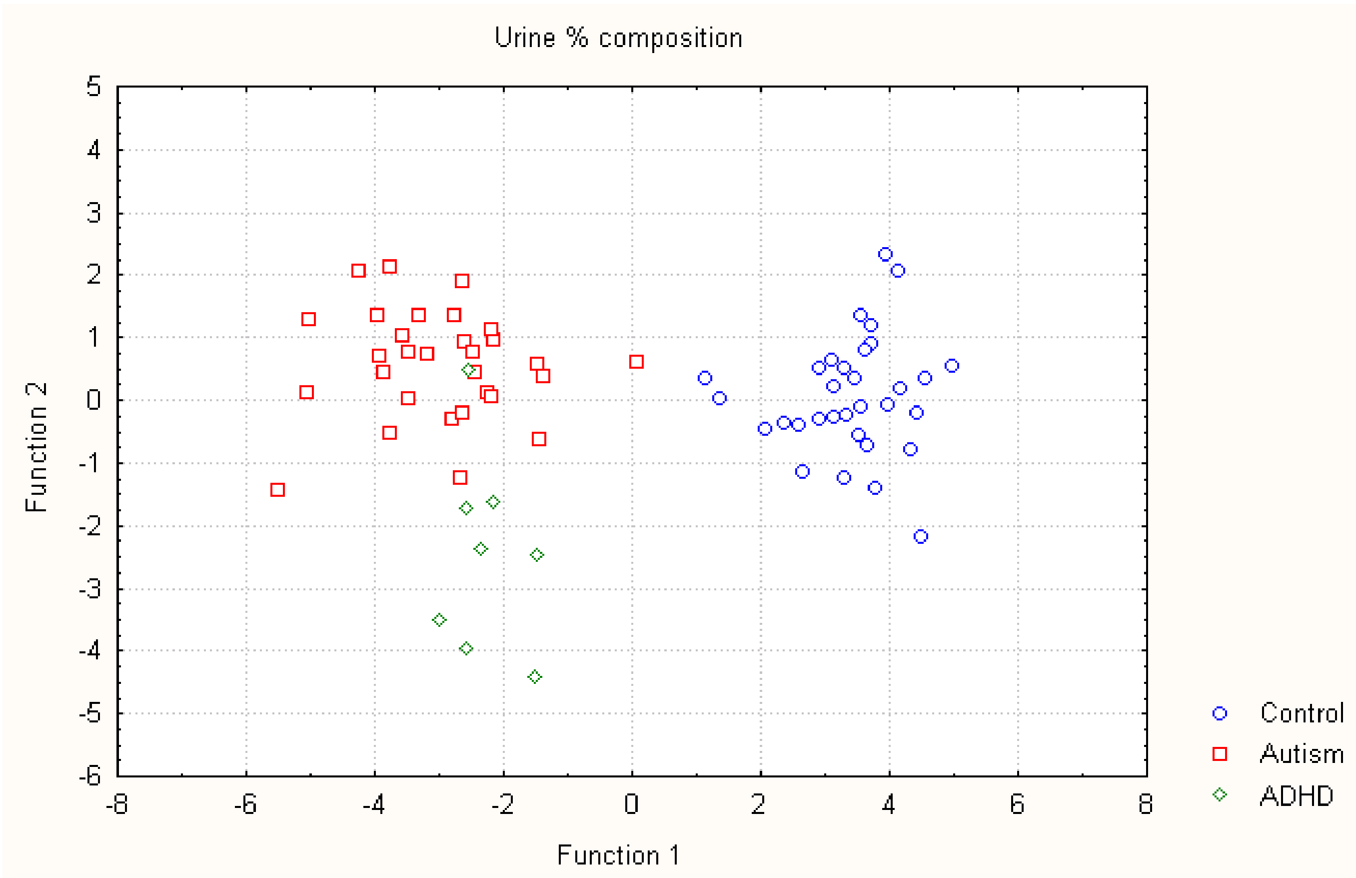


Aims and methods

- The aims and methods of the project were to obtain metabolic profiles of:
  - Urinary amino and organic acids by GCMS
    - HPLC method used to quantify urinary peptides of dietary and bacterial origin
    - HPLC method for characterisation of currently unidentified urinary molecules
  - Faecal microbiology through culture and PCR

Results

- The study recruited:
  - 35 children with autism
    - Analysed as 2 separate groups:
      - Those undergoing metabolic intervention (19)
      - Those without previous metabolic intervention (16)
  - 19 age and gender matched controls

Urine results

- Amino acid trends:
  - Low:
    - serine, alanine, glycine, valine, threonine, leucine

General Amino Acid Profile

	Patient Data	Average Value	low	average	high
serine:	3.97%	(9.16%)	●	○	○
alanine:	2.19%	(7.08%)	●	○	○
glycine:	5.77%	(20.81%)	●	○	○
valine:	0.52%	(1.31%)	●	○	○
threonine:	1.59%	(2.17%)	○	●	○
leucine:	0.16%	(0.48%)	●	○	○
proline:	4.16%	(2.66%)	○	○	●
asparagine:	0.71%	(1.52%)	○	●	○
hydroxy-proline:	2.88%	(0.55%)	○	○	●
aspartic acid:	1.16%	(1.66%)	○	●	○
phenylalanine:	0.62%	(1.05%)	●	○	○
ornithine:	1.20%	(0.56%)	○	○	●
glutamic acid:	8.98%	(9.89%)	○	●	○

○ *Amino acid profile type:* 4

Urine results

- High:
  - proline, hydroxyproline

Connective tissue amino acids					
lysine:	2.64%	(2.35%)	○	●	○
proline:	4.16%	(2.66%)	○	○	●
hydroxy-proline:	2.88%	(0.55%)	○	○	●

Urine results

- Organic acid trends:
  - High:
    - citric acid
  - Low:
    - Succinic acid

Organic acids					
phenylacetic acid:	0.51%	(1.19%)	○	●	○
succinic acid:	0.51%	(1.37%)	●	○	○
aconitic acid:	3.97%	(4.39%)	○	●	○
citric acid:	4.67%	(0.74%)	○	○	●

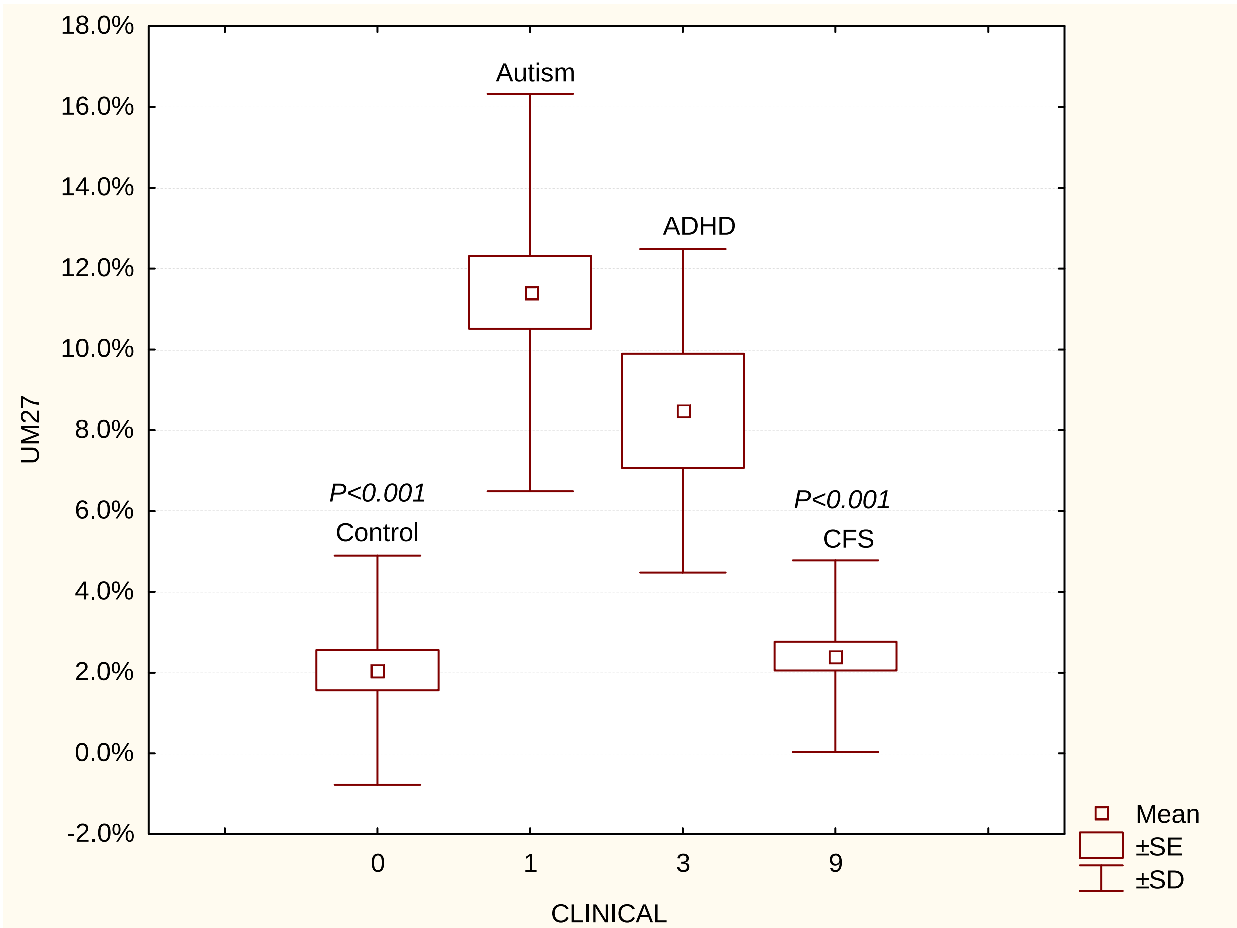
Urine results

- Unidentified urine metabolites:
  - High:
    - UM27 (has since been identified)
    - UM28

Unidentified Urine Metabolites

CFSUM1:	1.48%	(2.23%)	○	◉	○
UM13:	0.00%	(0.12%)	◉	○	○
UM13a:	0.22%	(0.16%)	○	◉	○
UM14:	0.00%	(0.20%)	◉	○	○
UM15:	0.00%	(0.05%)	◉	○	○
UM15a:	0.70%	(0.57%)	○	◉	○
UM17:	0.07%	(0.20%)	○	◉	○
UM27:	25.79%	(0.27%)	○	○	◉
UM28:	1.85%	(0.05%)	○	○	◉

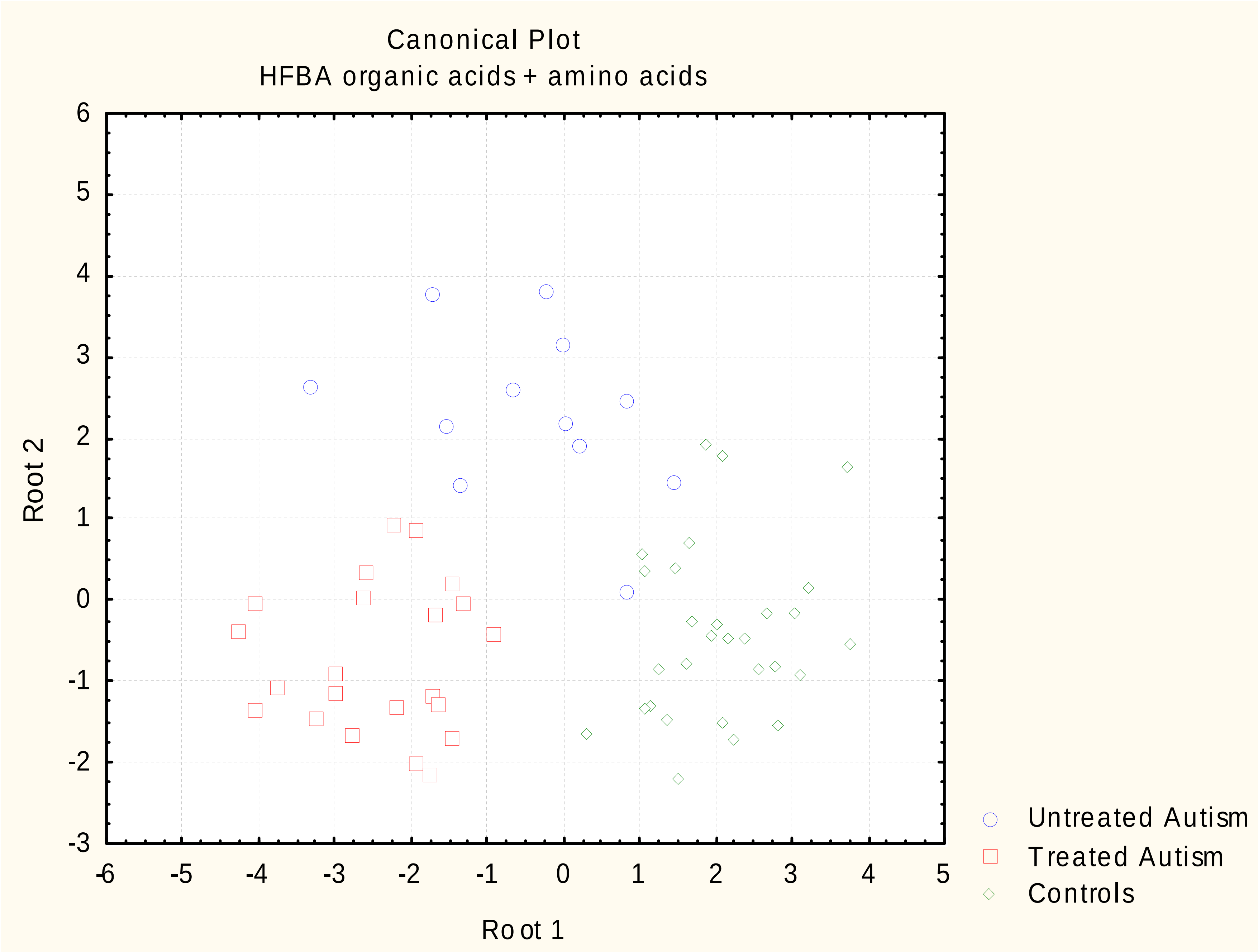
Autism: UM27



18.0%
16.0%
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
-2.0%
UM27
Autism
ADHD
P<0.001
Control
P<0.001
CFS
0
1
3
9
CLINICAL
Mean
±SE
±SD

Molecule characterisation

- Unidentified urinary metabolite 27: UM27
  - Autism group had six times the amount of UM27 as age and sex matched controls
  - Purification technique development using HPLC
    - Ion exchange LC
    - Reverse phase LC
  - Fragment of a larger molecule?
  - Dietary origin?
  - Marker of infection?
  - A dipeptide, Proline-Hydroxyproline
    - The only reference to this molecule in the literature relates to its use as a marker in bone resorption.


Canonical Plot
HFBA organic acids + amino acids
Root 2
Root 1
6
5
4
3
2
1
0
-1
-2
-3
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
Untreated Autism
Treated Autism
Controls

Summary

- AT and C similar profiles
- AU comparatively differences in:
  - essential amino acids
  - branched chain amino acids (Val, Leu, Ile)
  - connective tissue amino acids (Gly-Pro,Lys)
  - neurotransmitter pre-cursors (Phe, Tyr, Trp)
  - citric acid, succinic acid (TCA cycle)

Urine comments

- Catabolic state
- Require ions with 2^+ charge due to high excretion of citric acid (Borensztein P *et al.* 1989 Effects of citrate on urinary calcium excretion. *Mineral & Electrolyte Metabolism*. 15; 353-58)
- High pain thresholds
  - Tyrosine:leucine ratio
  - Body pain
- Evidence of infection?
- Molecules of dietary origin in the urine?

Faeces results

- Trends:
  - *E. coli* low or absent

Predominant Organisms	% total aerobic flora	Ranges
Eschericia coli:	6.25%	70-90%
Klebsiella/Enterobacter:	0.00%	<10%
Enterococcus:	0.00%	<5%
Staphylococcus:	0.00%	
Others:	93.750%	

Faeces results

- Bacteroides high
- Bifidobacterium:
  - 1.52 x 10^9 in control children
  - 0.8 x 10^9 in children with autism
- Study groups were well separated by discriminant function analysis
  (Wilks' Lambda 0.25983, P<0.0002)

Predominant Organisms	% total anaerobic flora	Ranges
Bacteroides:	99.09%	90-95%
Eubacteria:	0.000%	
Bifidobacterium:	0.755%	5-11%
Lactobacillus:	0.000%	0-1.3%
Clostridia:	0.076%	1-10%
Others:	0.076%	

Faeces results

- Long chain fatty acids high – fat malabsorption

LIPID SUMMARY

%Sterols:	7.49%	●	○	○	*(18.9%)*
% Fatty Acid Ethyl Esters (FAEE):	1.17%	○	●	○	*(6.6%)*
% Fatty Acids (FA):	91.29%	○	○	●	*(76.1%)*
FAEE / FA:	0.01	○	●	○	*(0.11)*
Sterols / (FA+FAEE):	0.081	○	●	○	*(0.32)*

Faeces results

- Presence of bacteria from body systems other than the gastrointestinal system

Other bacteria detected:

Aerobes: non haemolytic Streptococcus species, alpha haemolytic Streptococcus species

Anaerobes:

Faeces comments

- Gut dysbiosis
  - Presence of ENT bacteria
- Low or absent *E. coli*
  - Serine production
  - An essential nutrient; a new vitamin?
- Fat malabsorption
  - Immune system
  - Cell to cell communication

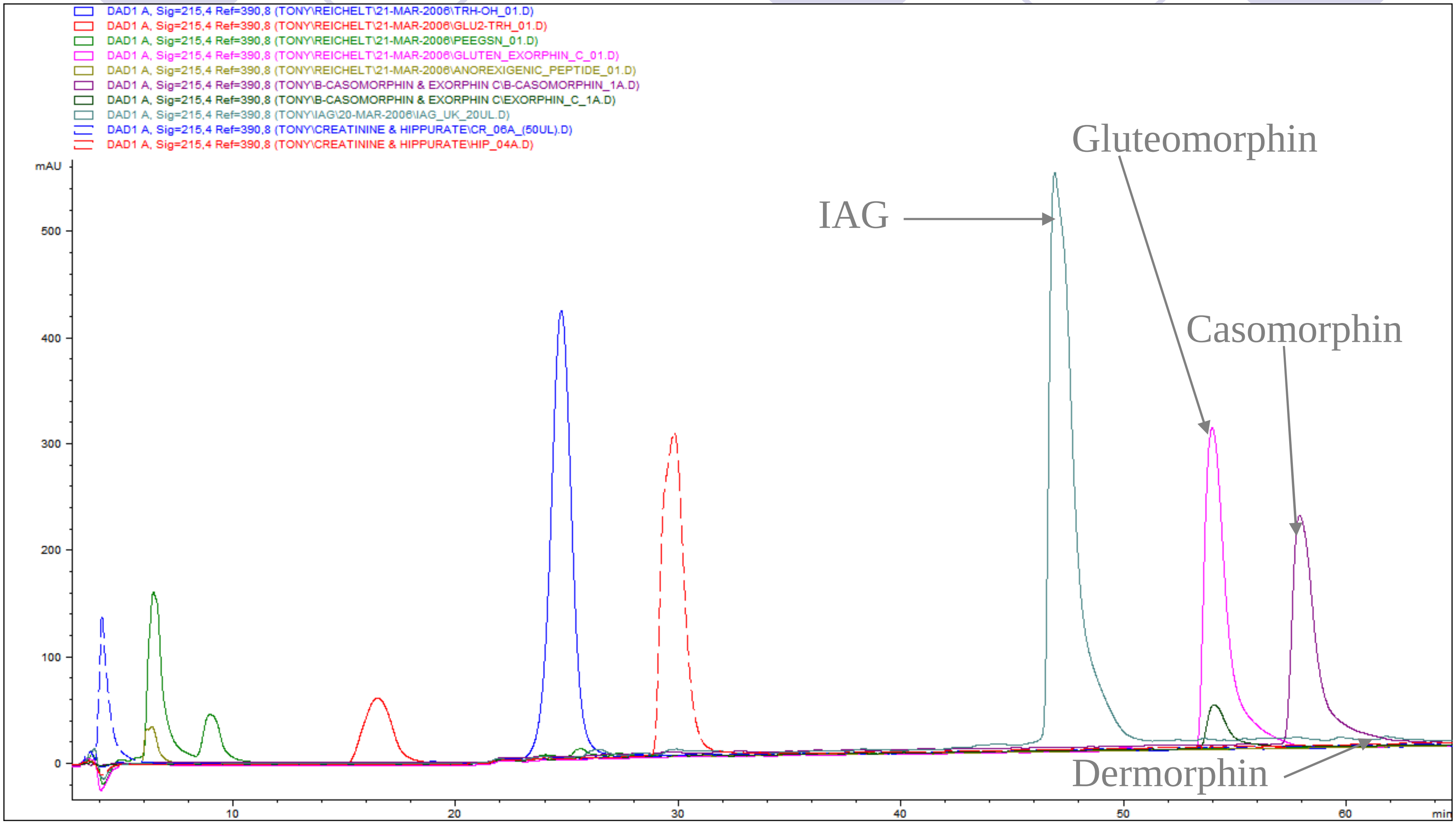
Urinary peptides

- Beta-casomorphine:
  - Tyr-Pro-Phe-Pro-Gly-Pro-Ile from milk
- Gluten exorphin C:
  - Tyr-Pro-Ile-Ser-Leu from wheat
- Dermorphin (wild South American poison dart frogs)
  - Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser
- Associated with brain irritation, brain fog, high pain thresholds
- Elute between 17 and 30 minutes on HPLC machine using current method
- Assay development
- Digestion by enzymes from gut villi or bacteria

Bacteria tested for ability to digest exorphins

- Indigenous gut flora
  - Able to breakdown β-casomorphin, gluten exorphin-C
- Other bacteria were derived from fermented foods
- Derived from raw unpasteurised milk
- Incubated the pure exorphin peptides with the bacteria for 4 hours at 37°C
- Separated the exorphins on HPLC
- Looked for decrease in peak size compared to controls

Typical HPLC Run to separate Exorphins

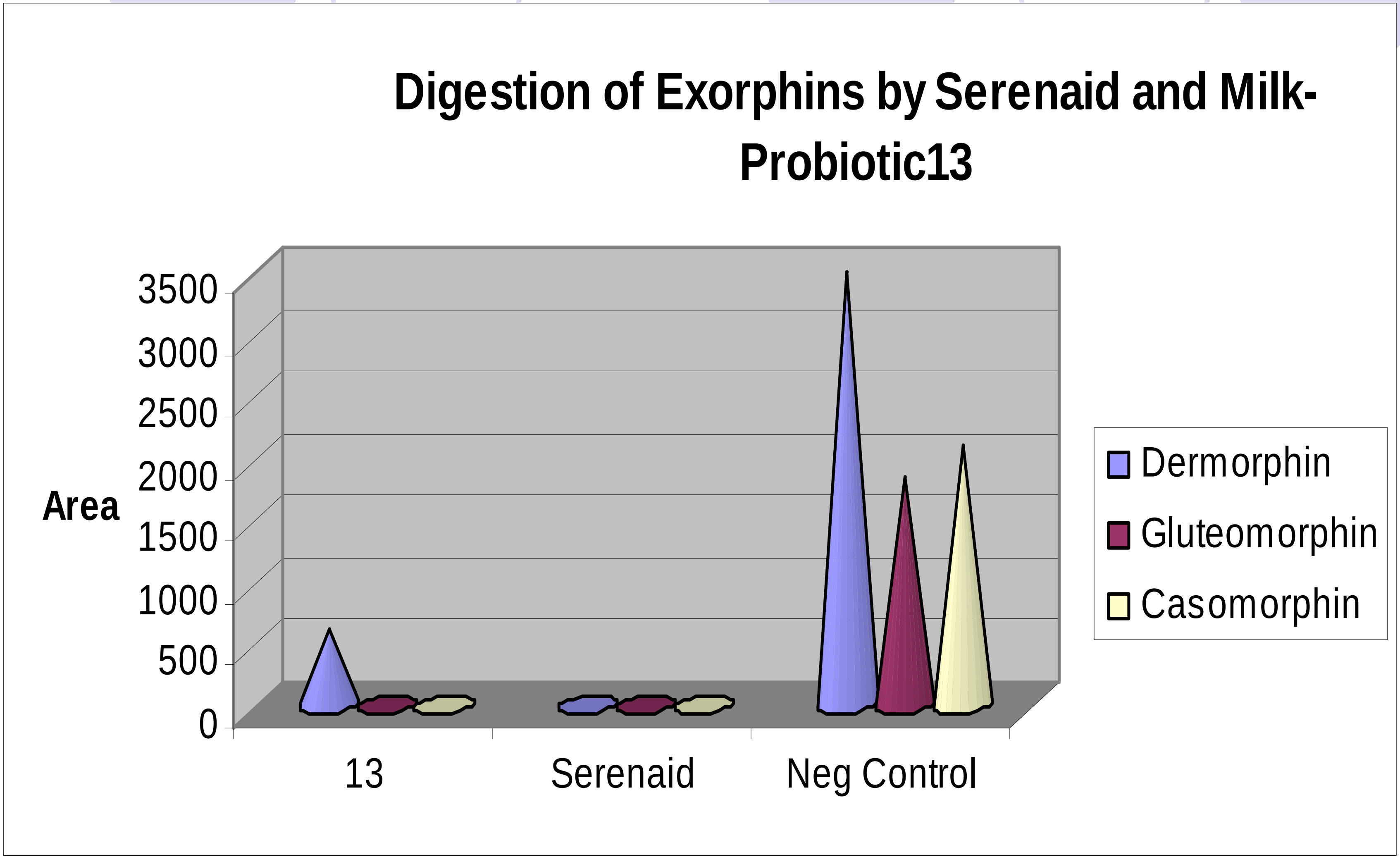


Two different samples of gluteomorphin have the same elution characteristics

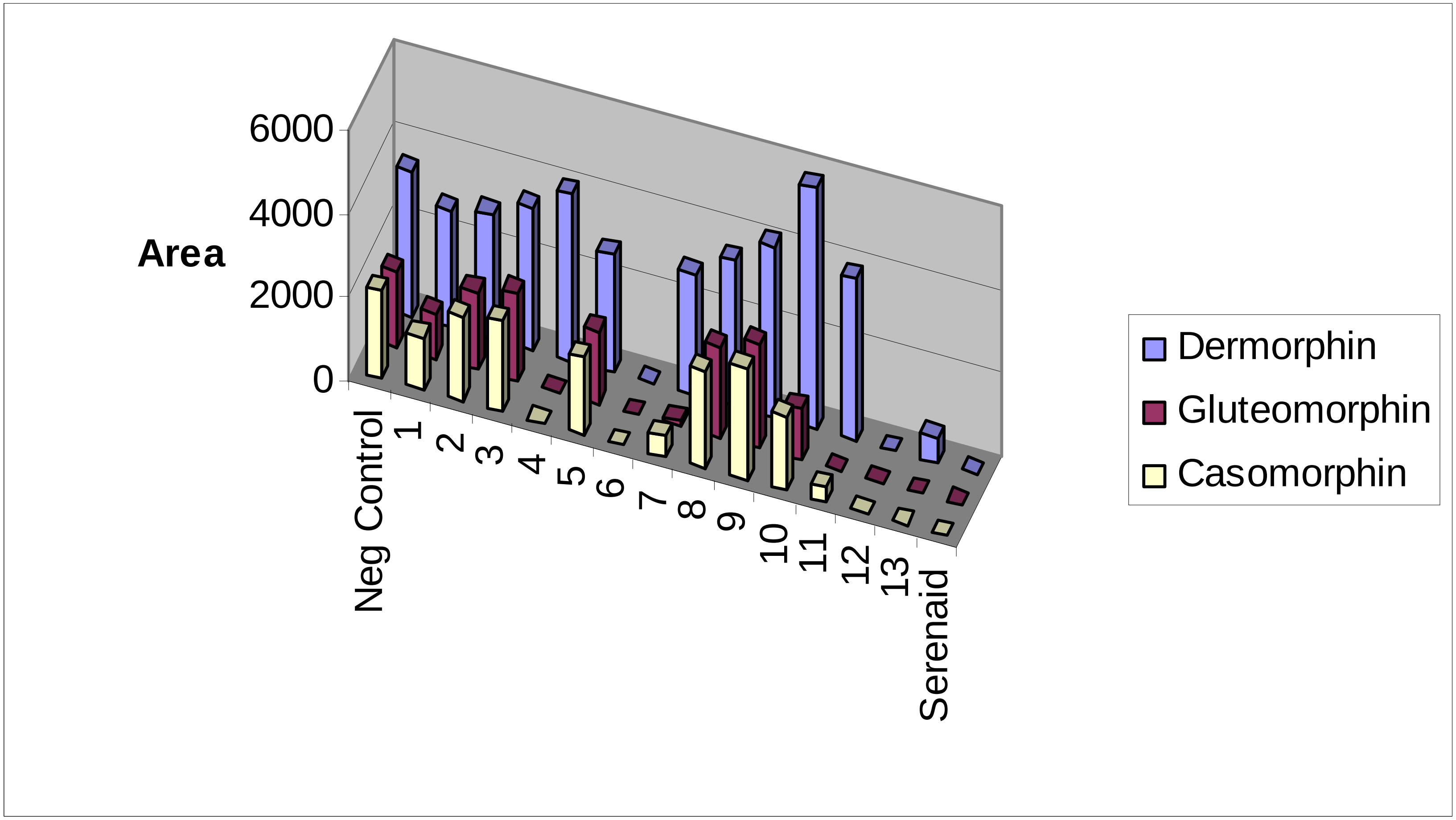
Dermorphin elutes at the position shown but was not included on this run.

Digestion with Serenaid

- Proprietary blend of six plant derived enzymes
- Can breakdown casomorphins and gluteomorphins
- High in Dipeptidyl peptidase IV activity
- Demonstrated digestion of dermorphin


Digestion of Exorphins by Serenaid and Milk-Probiotic13
Area
3500
3000
2500
2000
1500
1000
500
0
13
Serenaid
Neg Control
Dermorphin
Gluteomorphin
Casomorphin

Results of incubation with bacteria from fermented foods



Role of diet in children with autism

- Gluten free, casein free diet (Knivsberg AM, Reichelt KL & Nodland M (2001) Reports on Dietary Intervention in Autistic Disorders. *Nutritional Neuroscience* 4: 25-37)
- Removal of other food sensitivities
  - Eg. High phenolic foods
- Improvements in:
  - Gut function
  - Speech
  - Learning
  - Eye contact
  - Social skills

Biomedical interventions

- Dietary change
  - Avoidance of potential allergic foods
  - Potentially minimise phenolic foods eg. tomatoes
    - Red ears after eating; flushed face
  - Downregulate potential for autoimmunity
- Fat in the diet; coconut; grass fed meat
- Essential fats, including cod liver oil and explore evening primrose oil
  - Supplementation often necessary due to long term malabsorption
- Minimise gut irritation; often no fruit until the gut flora improve; sugars feed the "bad" bugs
- Add nutrients; often P5P, Mg, zinc
  - Zinc is responsible for the taste and texture of food
  - Promote metallothionein

Dietary Change

- Step 1:
  - Gluten and casein free diet trial
    - Includes chocolate and cocoa
  - Initially for 4 weeks
  - Review compliance
  - Encourage continuation
  - Must be super strict for the trial period (and ongoing)
  - Important to determine if the whole family are taking part or just the child with ASD
  - Important to explain WHY these exclusions are necessary
    - Helps compliance enormously

Dietary Change

- Step 2:
  - Low phenolic trial
  - Depends on parents but this can also be done at the same time as step 1 if necessary
  - Initially no red food
    - Tomato (and tomato products)
    - Capsicum
    - Apple
    - Chilli
    - Strawberry etc.
  - Warranted if red ears, cheeks after eating
  - Encourage epsom salts baths

Dietary Change

- Step 3:
  - No or low fruit
  - Can also be done earlier depending on family situation
  - Trial with pears only
  - Omit pears if necessary
  - Possible fructose malabsorption
  - Possible food for bad bugs
    - We effectively starve them
  - Possible reduction of phenolic component of the diet

Dietary Change

- Step 4:
  - No refined sugar and no “white” GF carbs
  - Also observe responses to honey and other natural sweeteners
  - Stevia usually a good option for baking
  - Don’t just replace wheat four with white rice flour (for example)
  - Encourage use of buckwheat flour, almondmeal etc for baking
  - Whole foods

Dietary Change

- Additional options:
  - The Specific Carbohydrate Diet is excellent
  - Diet based mostly on meat, fish, eggs, vegetables, nuts, low sugar fruits
  - The only carbohydrates allowed are monosaccharides
    - Easily absorbed across the intestinal wall
    - No disaccharides or polysaccharides
  - Purpose is to change the gut bacteria and limit gut wall irritation/inflammation
  - Some children respond to this quickly and distinctively
  - Watch oxalate reactions from higher nut and nut flour consumption
    - Eg. Urinary problems
    - Continued malabsorption even with other dietary changes

Dietary Change

- Body Ecology Diet
  - Focus on the inclusion of fermented foods such as sauerkraut and coconut kefir
  - Again, focus on changing gut bacteria profiles
  - Positive and negative reactions to the fermented foods
  - Die off?
  - Different approaches work for different children

Biomedical interventions

- Gut as barometer
- Stabilise behaviours; improvement in ASD symptoms
- Add herbs
  - Phase 1 and 2 liver herbs
  - Improve PST pathway
  - Anti "bug" herbs
  - Adrenal support
- Chelation

Evolutionary aspects

Some of humanity's most baffling "non-infectious illnesses" may turnout to be caused by pathogens

- eg *Helicobacter pylori* → gastric ulcers
- eg *Chlamydia* → heart disease
- eg HPV → cervical cancer
- "Nanobacteria" → kidney stones
- Mycoplasma → rheumatoid arthritis
- Streptococcus → ASD???

To conclude…

- Individualised advice
- Maximise nutrition
- Practical advise
- Recipe and/or meal plan provision
- Communication
- Hope
- Support from outside
- Nourishment for body, mind and soul

Autism

- Multisystem metabolic disorder
- Answers lie in individual cases
- Path to a diagnosis of ASD often different for each child
- Research must investigate subgroups based on symptoms &/or time of presentation of symptoms, rather than simply the “label’ of ASD

Hope for intervention
Hope for prevention

Contact details

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