Measuring Microbiome Metabolomic Profiles across Plasma, Skin strips, Interstitial Fluid and Distal Small Intestinal Fluid

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- Human Metabolome Technologies
- VP and Sr. Analyst

Claims

- #1 We can learn much from the study of healthy people
- #2 Microbial metabolites extend beyond the usual suspects
- #3 A systems biology approach is necessary for personalized medicine

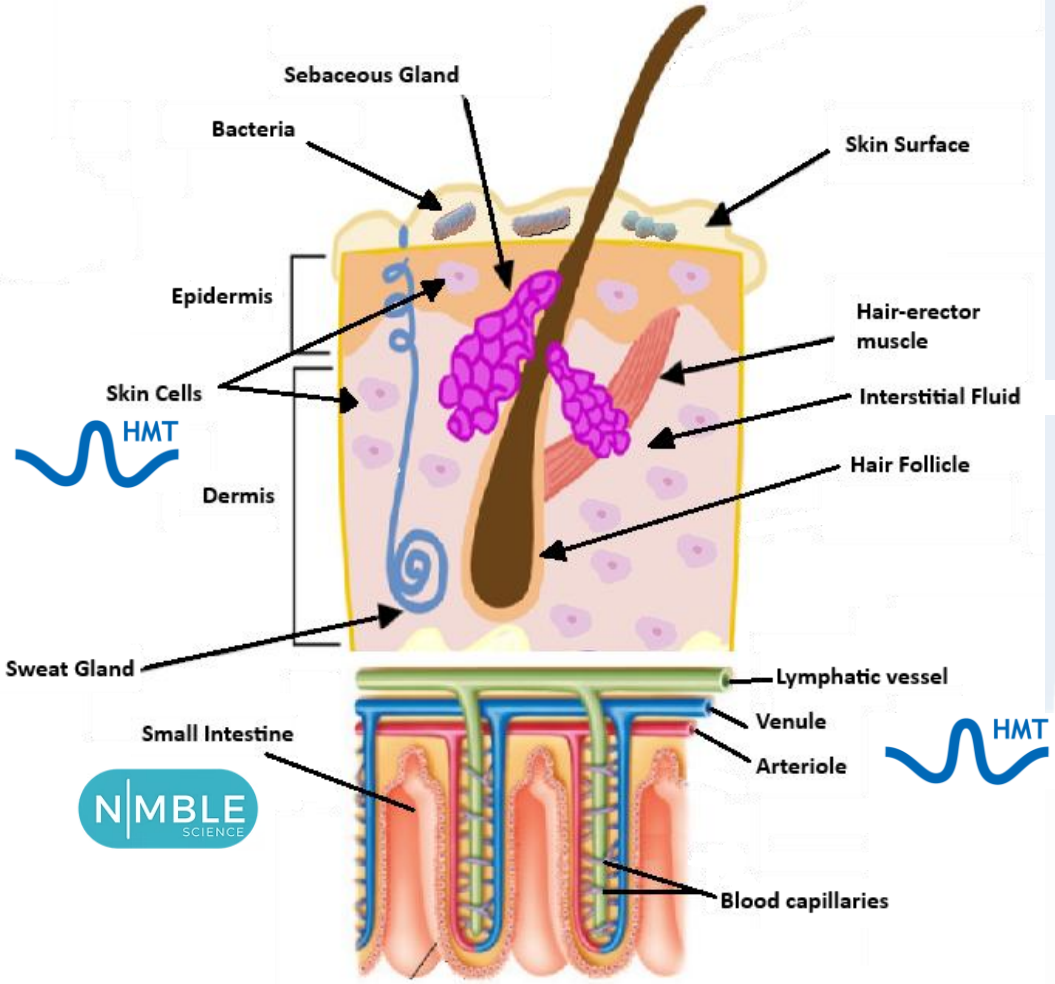
Speaker Bio

- 1980-1984 Staff Fellow NIH Bureau of Biologics (CBER)
- 1984-2002 Volwiler Research Fellow & Head Structural Chemistry Abbott Labs (Abbvie)
- 2002-2012 Sr. Director Translational Medicine Biogen
- 2012 – Today VP & Sr. Analyst Human Metabolome Technologies - America
- Board Member Metabolomics Society

Examine these 4 compartments and connections to microbiome

- Plasma
- Skin strips
- Interstitial fluid
- Distal small intestinal fluid

A communication network



Skin – (epidermis and dermis)

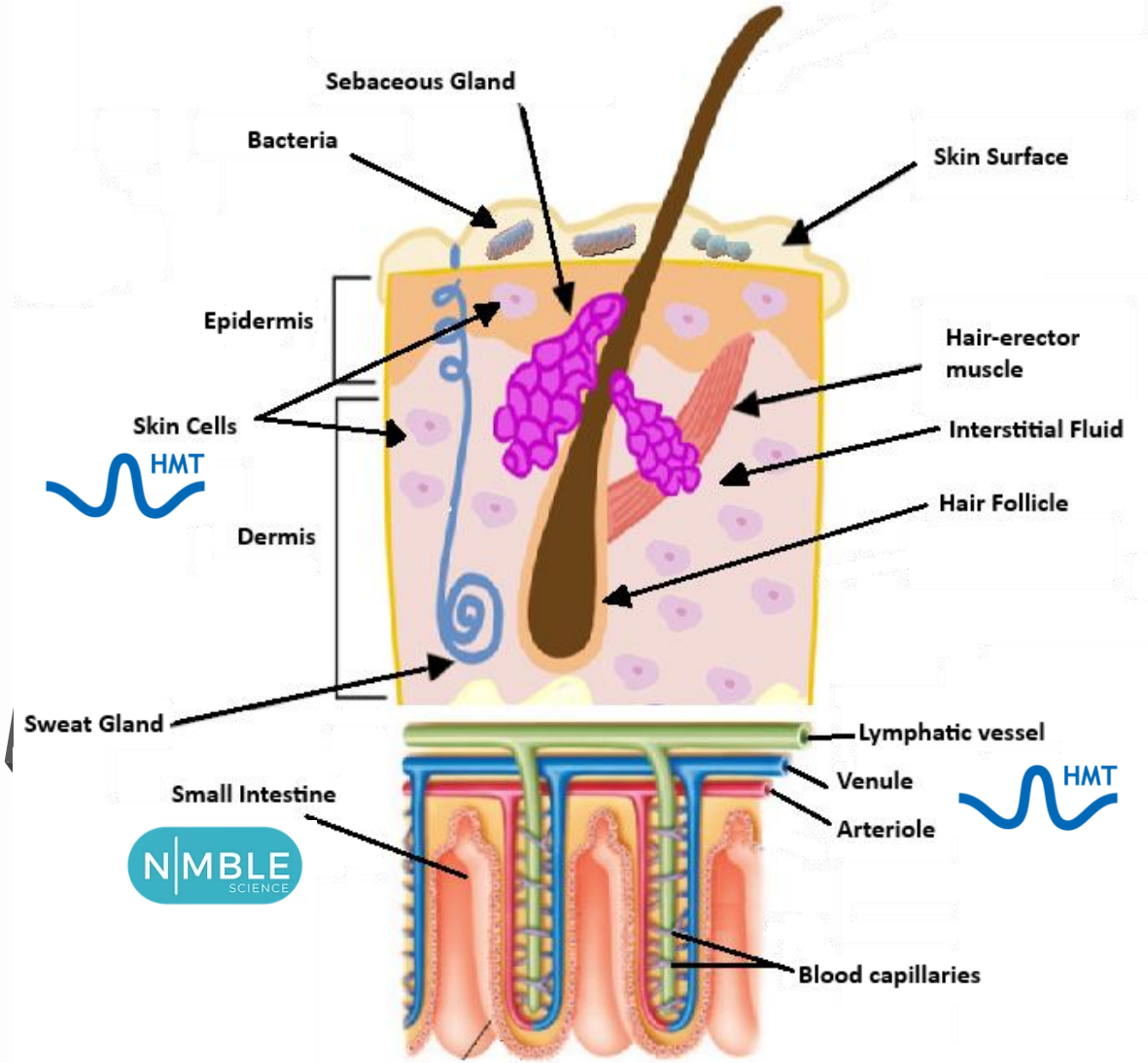
ISF ascilion

Plasma

Distal small intestine NMBLE SCIENCE

Q: How do their microbial metabolic profiles compare?

The microbiota



Surface of skin
Hair follicles
Sweat glands/Sebaceous glands
Distal small intestine

Q. How different are the metabolic profiles in skin and intestine?

Q. Are these profiles shared with interstitial fluid and plasma?

Data Acquisition

- **Skin Strips**: OMEGA Scan and LC-OMEGA
- **Skin Interstitial fluid**: OMEGA Scan and LC-OMEGA
- **Plasma**: OMEGA Scan, LC-OMEGA and MSCAN
- **Distal small intestinal fluid**: OMEGA scan

OMEGA Scan

- Capillary Electrophoresis FTMS untargeted
  - High resolution separation High resolution / High sensitivity MS
  - Focus Polar metabolome
  - Annotation library (Tier one identifications)
  - Unidentified list (m/z, peak area, R.T.) annotated using OMEGA Search program
  - Together 600 to over 900 polar metabolites in biofluids
- Q353 / Q110 targeted
  - Quantitative (uM) 353 or 110 polar metabolites

LC-OMEGA

- HPLC-FTMS
  - High sensitivity MS
  - Affinity removal of complex lipids and Phospholipids to increase sensitivity
  - Annotation library (Tier one identification)
  - Unidentified list (m/z, area, R.T.) identified using OMEGA Search program
  - Together over 300 to over 700 lipids in biofluids

MSCAN

- LCMS/MS
- 400 targeted lipid mediators
- Acyl carnitines – Lysophospholipids – Ceramides – Fatty acids – Gangliosides - Sphingolipids
- Focus Inflammatory – Immune – Lipid regulation
- Typically, >260 – 280 in healthy human plasma

Virtual Human Plasma Metabolome
Tracking 1420 plasma metabolites

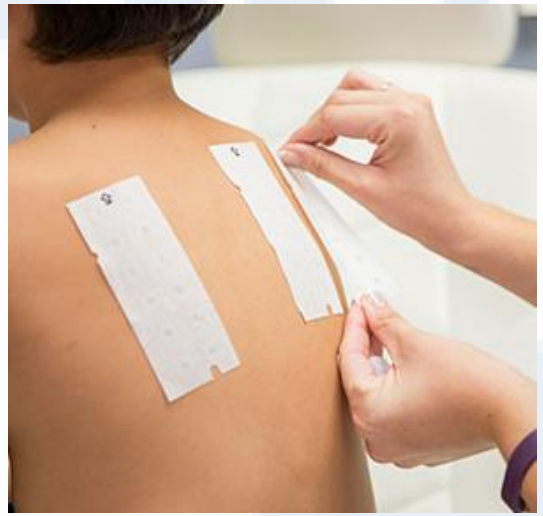
- 745 polar metabolites (OMEGA Scan)
- 353 quantitated polar metabolites (Q353) - uM
- 275 lipids (LC-OMEGA Scan)
- 400 Lipid mediators (M-Scan)

- Data from over 400 healthy plasma

- Webinar on Virtual Human Plasma last month

Skin metabolites

- HMT Skin Strips
  - Tape #2-3 (epidermis) to #6-8 (dermis) duplicates
    - One for Polar extraction
    - One for Lipid extraction
  - HMT – Protocol for careful sampling and shipping
  - **Over 1,800 annotations in skin library**

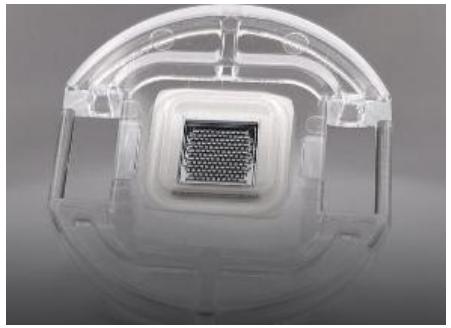


D100 - D-Squame Standard Sampling Discs

Interstitial fluid (ISF) Metabolites

ascilion

- Automated device
- Takes about 30 minutes to remove ~ 20-50 ul ISF (x5 dilution)
- Painless
- **Over 1,000 annotations**



Distal Small Intestine Fluid Metabolites

- SIMBA capsule
- Swallow capsule – opens in distal small intestine
- Capture 50 to 100 ul fluid
- Collect capsule – remove fluid - < 20 ul analysis
- NIMBLE here today – see their exhibit for more details
- **Over 900 annotations (Polar only)**

NIMBLE SCIENCE

SIMBA Capsule

Small
Intestine
Micro**B**iome
Aspiration
Capsule

What are microbial metabolites?

- More than usual suspects: SCFAs, indoles, TMAO,,,,,

Expand our range on microbial metabolites
Non-conventional metabolites

- Amino Acids
- Organic acids
- Cyclodipeptides
- Gamma Glu dipeptides
- Acetylated amino acids
- Conjugated amino acids
- Fructosylated amino acids
- Small peptides
- Phase 2 metabolism
- Others?

Compare potential 8 microbial metabolite classes across all 4 matrices

1. Amino Acids
2. Gamma Glu amino acids
3. Fructosylated amino acids
4. Glycine conjugates
5. Microbial
6. Oxidative metabolites
7. Small peptides
8. Caffeine metabolites

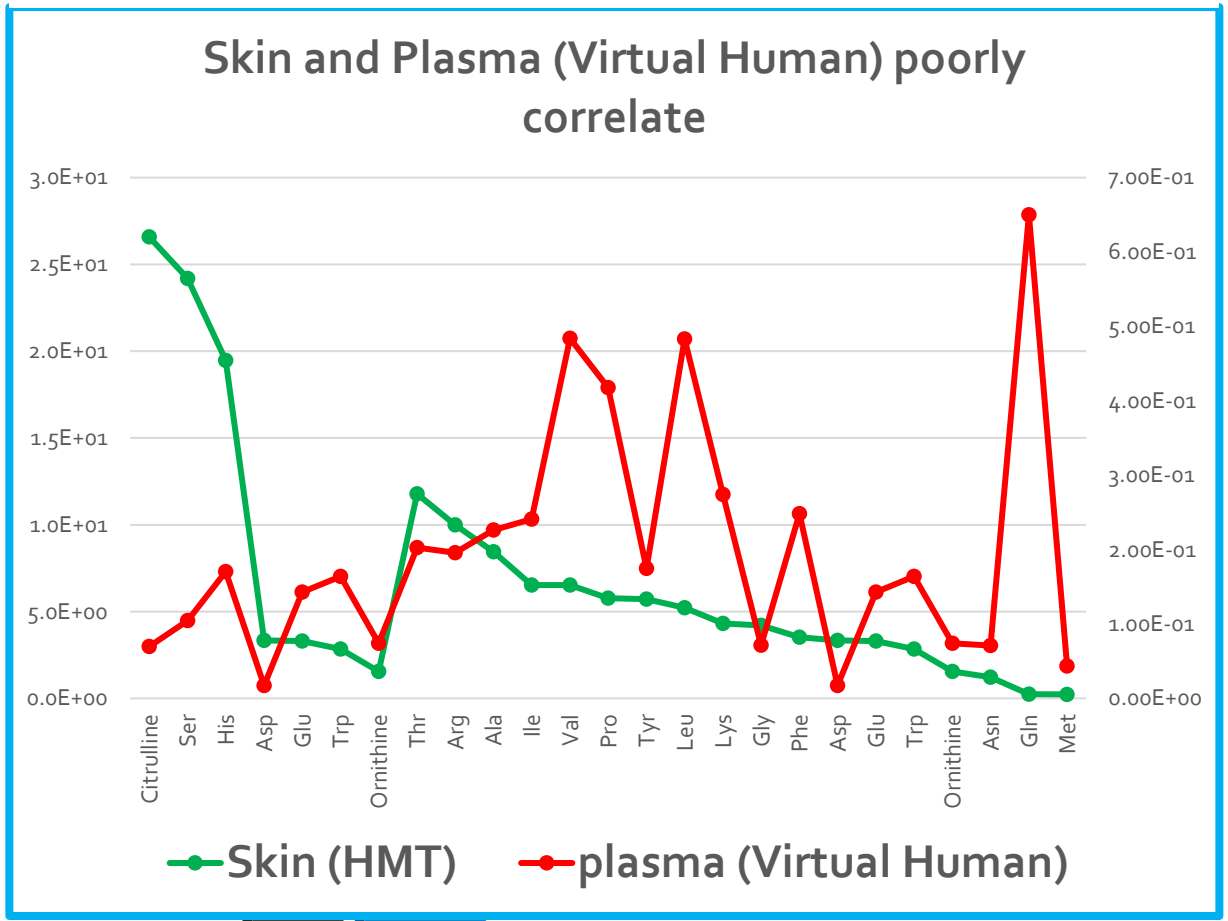
7TH SKIN MICROBIOME & COSMECEUTICALS

25 Amino Acids

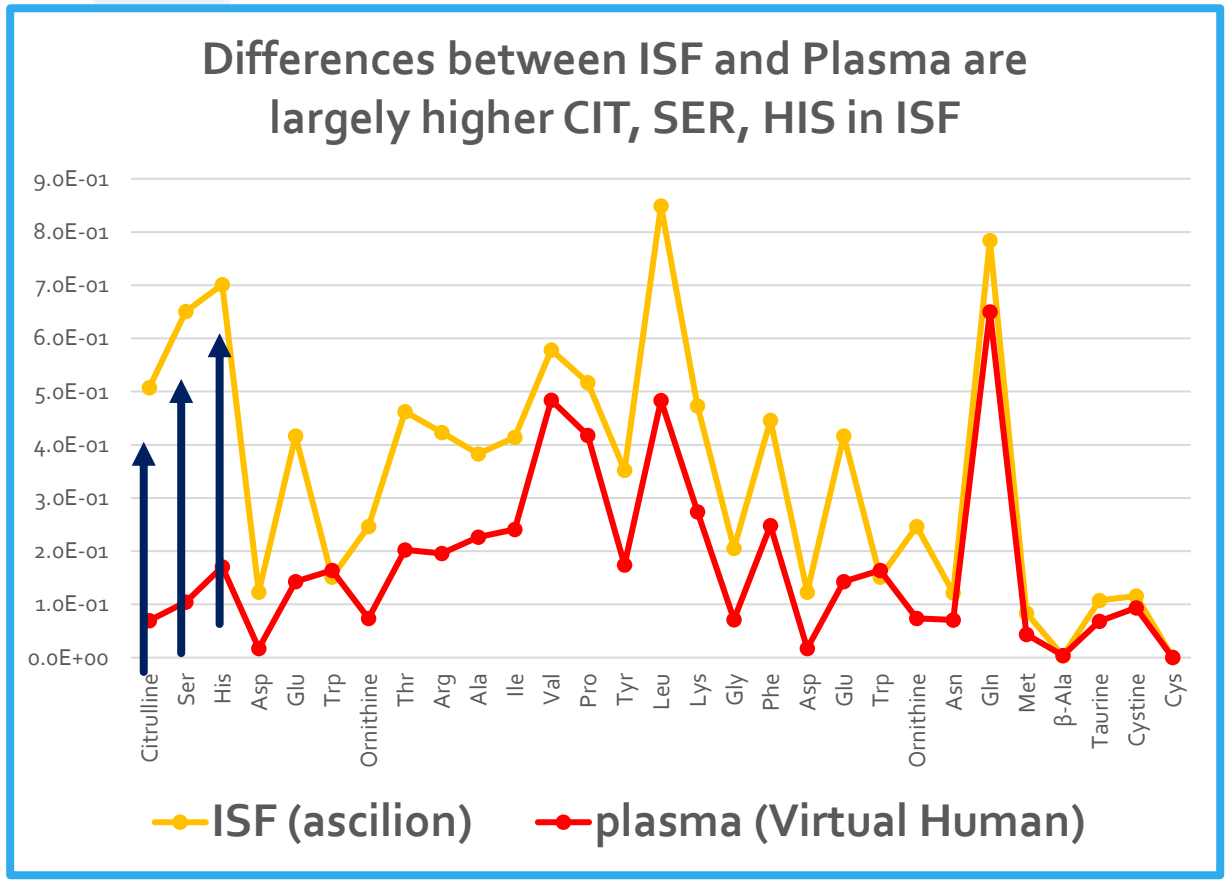
Sorted high to low in Skin

	Skin (HMT)	ISF (Ascilion)	Plasma (Virtual Human)	Distal small intestine (Nimble)
Citrulline	2.7E+01	5.1E-01	6.95E-02	7.16E-02
Ser	2.4E+01	6.5E-01	1.05E-01	1.75E+00
His	1.9E+01	7.0E-01	1.71E-01	1.20E+00
Thr	1.2E+01	4.6E-01	2.03E-01	2.12E+00
Arg	1.0E+01	4.2E-01	1.96E-01	2.81E+00
Ala	8.4E+00	3.8E-01	2.26E-01	1.58E+00
Ile	6.5E+00	4.1E-01	2.41E-01	3.99E+00
Val	6.5E+00	5.8E-01	4.84E-01	4.55E+00
Pro	5.8E+00	5.2E-01	4.18E-01	3.17E+00
Tyr	5.7E+00	3.5E-01	1.74E-01	2.71E+00
Leu	5.2E+00	8.5E-01	4.83E-01	8.94E+00
Lys	4.3E+00	4.7E-01	2.74E-01	2.69E+00
Gly	4.2E+00	2.1E-01	7.14E-02	7.40E-01
Phe	3.5E+00	4.5E-01	2.48E-01	4.54E+00
Asp	3.3E+00	1.2E-01	1.73E-02	1.36E+00
Glu	3.3E+00	4.2E-01	1.43E-01	1.99E+00
Trp	2.8E+00	1.5E-01	1.64E-01	1.33E+00
Ornithine	1.6E+00	2.5E-01	7.40E-02	1.48E-03
Asn	1.2E+00	1.2E-01	7.10E-02	1.52E+00
Gln	2.4E-01	7.8E-01	6.50E-01	1.47E+00
Met	2.3E-01	8.4E-02	4.37E-02	5.35E-01
β-Ala	1.1E-01	3.5E-03	3.99E-03	0
Taurine	1.8E-02	1.1E-01	6.8E-02	4.03E-02
Cystine	1.8E-02	1.2E-01	9.35E-02	7.91E-01
Cys	7.6E-03	9.3E-04	1.70E-04	5.20E-03

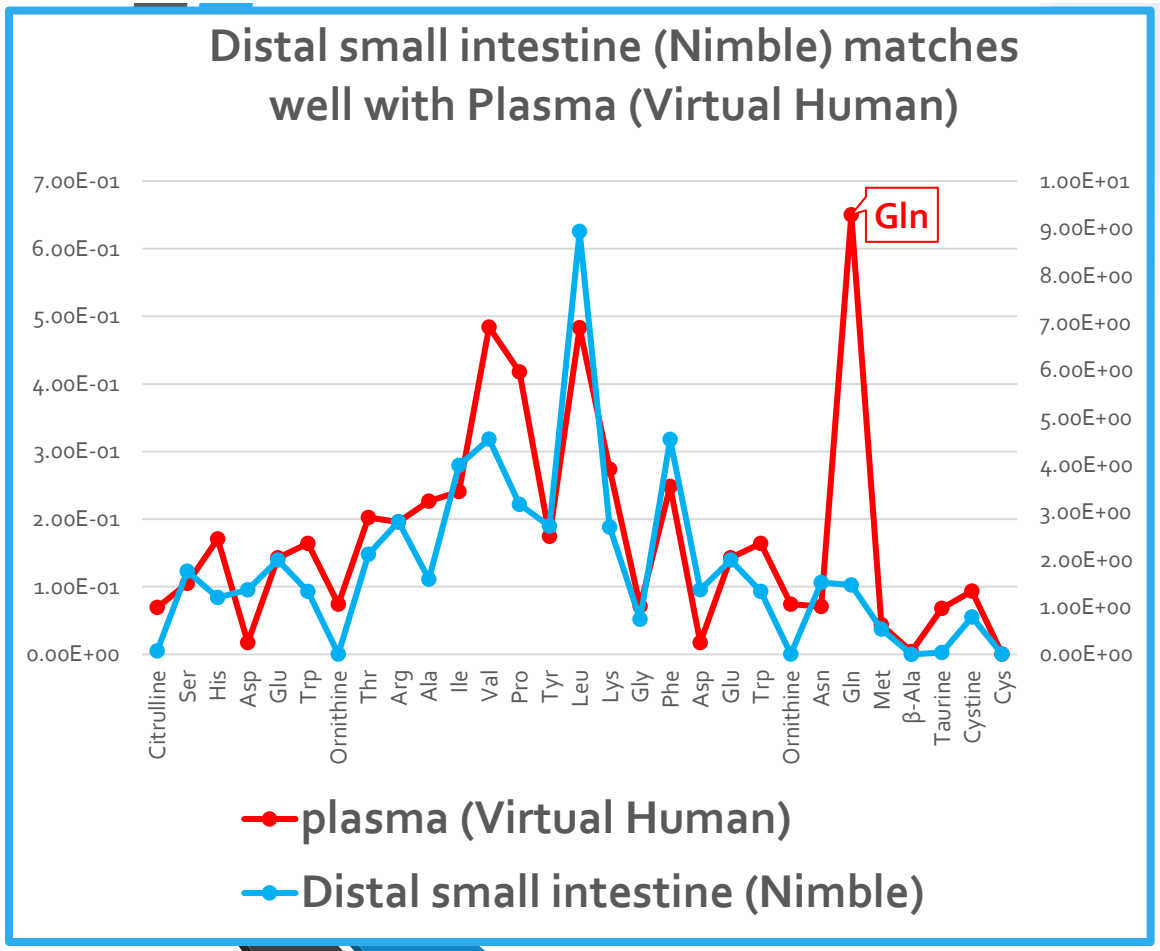
Skin AA and plasma AA poorly correlate



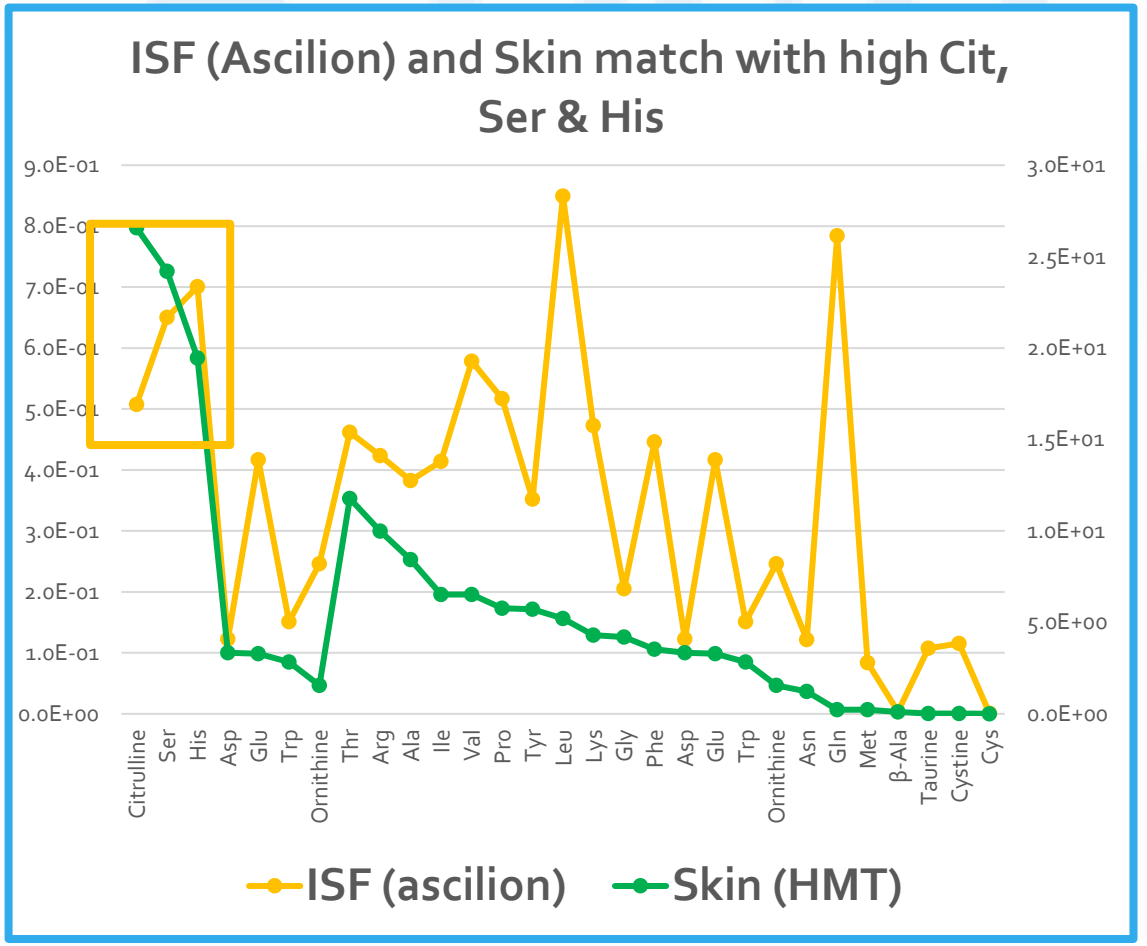
ISF and Plasma show nice correlation; differ Cit, Ser, His



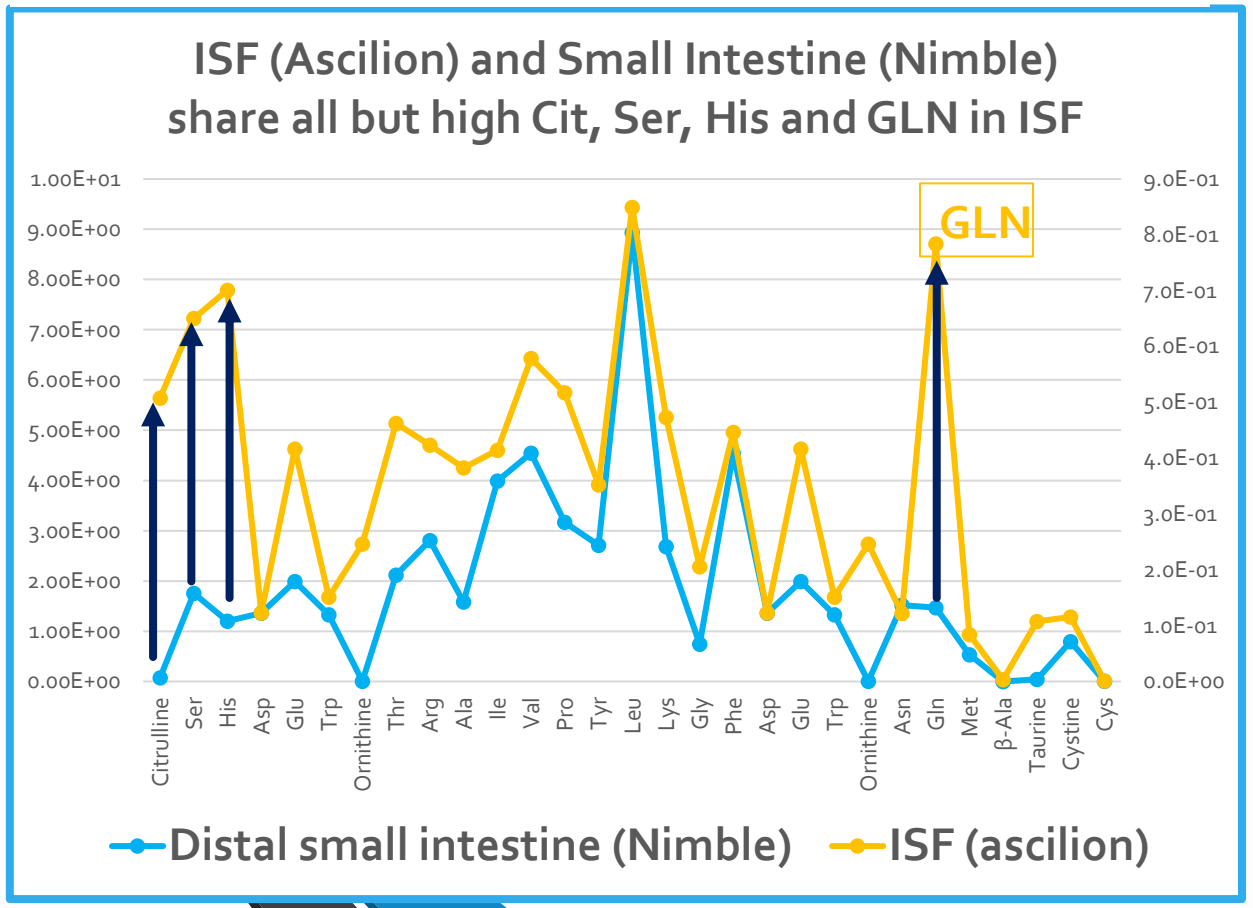
Small Intestine and Plasma AA match; differ GLN



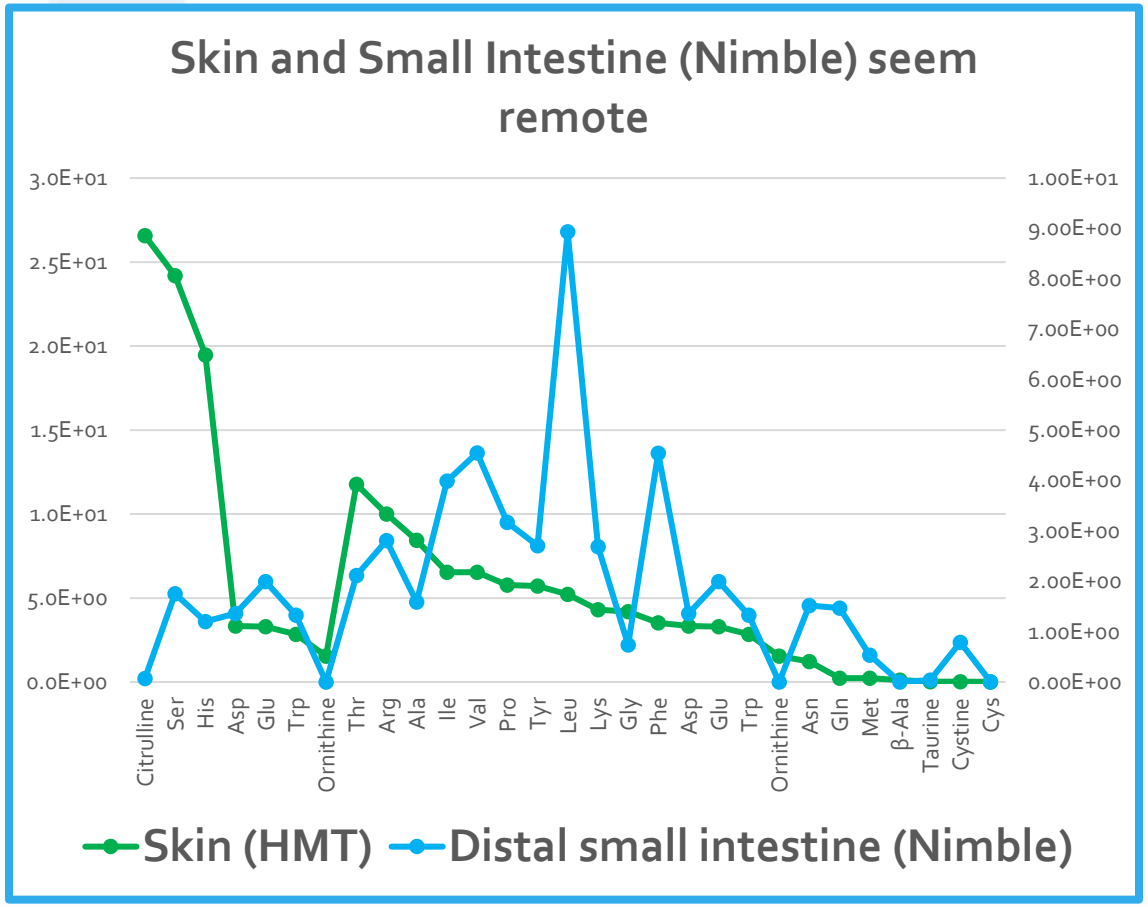
ISF and Skin share high levels of CIT, SER, HIS



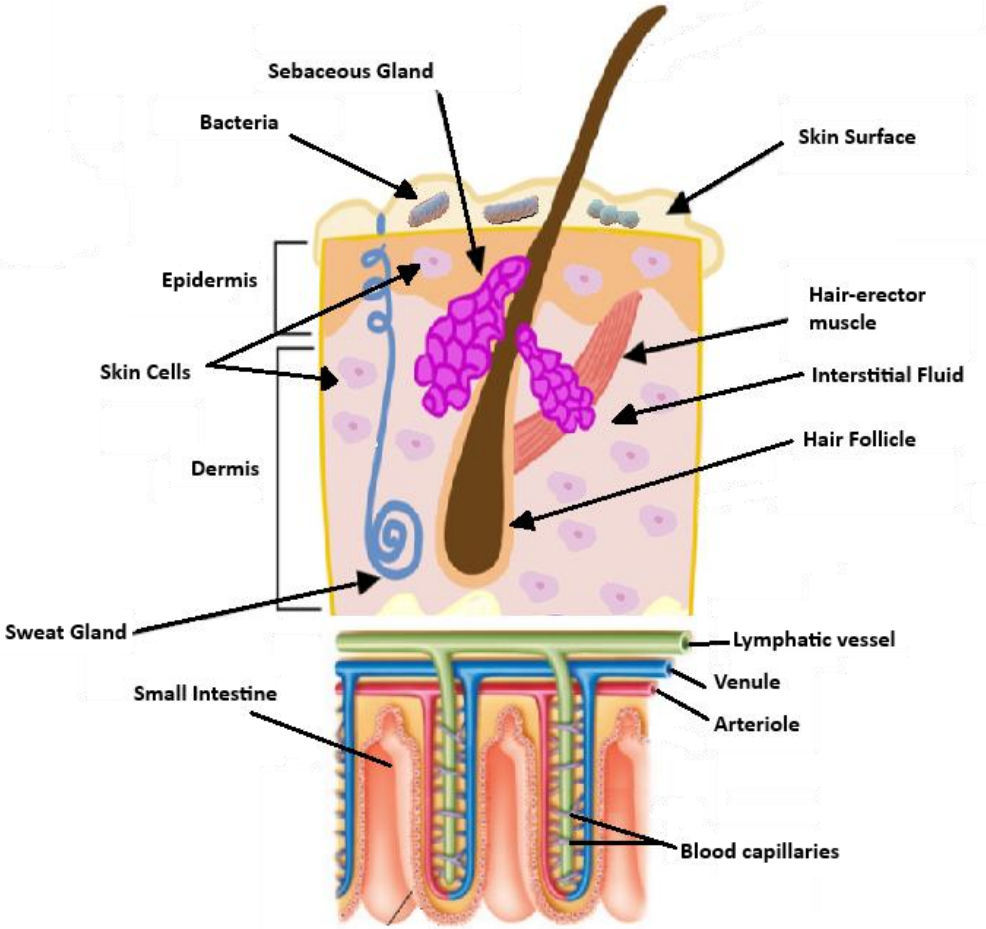
ISF and Small Intestine show correlation; differ high GLN, CIT, SER, HIS



Skin and Small Intestine are dissimilar



Summary of Amino Acid comparisons



Skin seems unique composition with CIT HIS SER as top amino acids

ISF – Plasma – Small Intestine ALL correlate well with exceptions

a. ISF has high CIT HIS and SER vs Plasma and Intestine

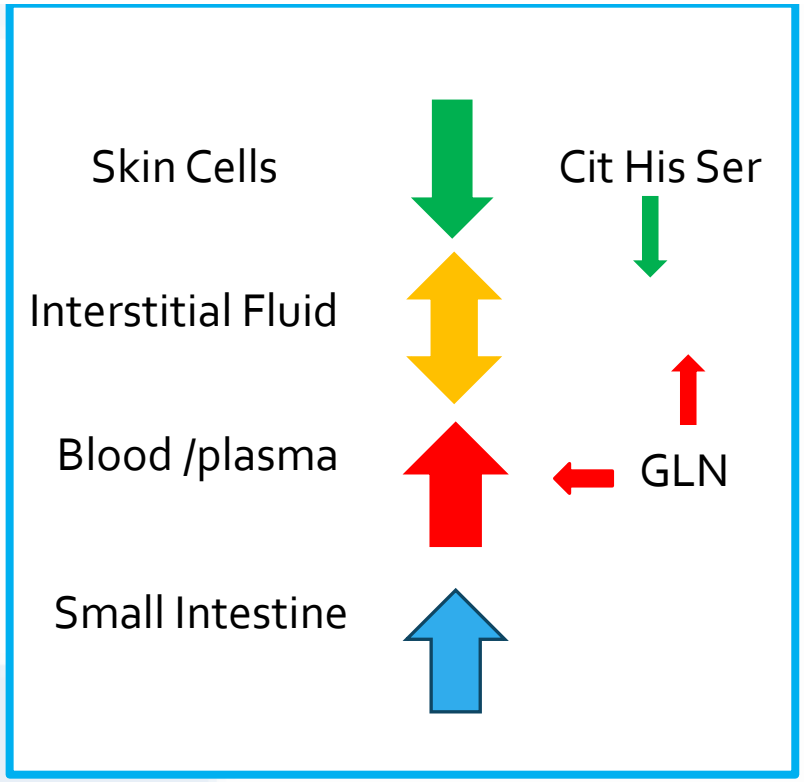
b. ISF and Plasma have high GLN vs Intestine

Plasma amino acids determined by distal small intestine while skin amino acids are locally controlled. Liver may be gatekeeper…

1. Amino acids transfer from the distal small intestine into the blood stream. These amino acids in blood are transferred into the skin interstitial fluid.

2. Glutamine is synthesized in skeletal muscle & adipose tissue *added to blood circulation and then transferred to skin interstitial fluids.*

3. Citrulline, histidine, and serine are highly concentrated in skin cells because they are key components of the natural moisturizing factor (NMF) – *these transferred to interstitial fluid.*

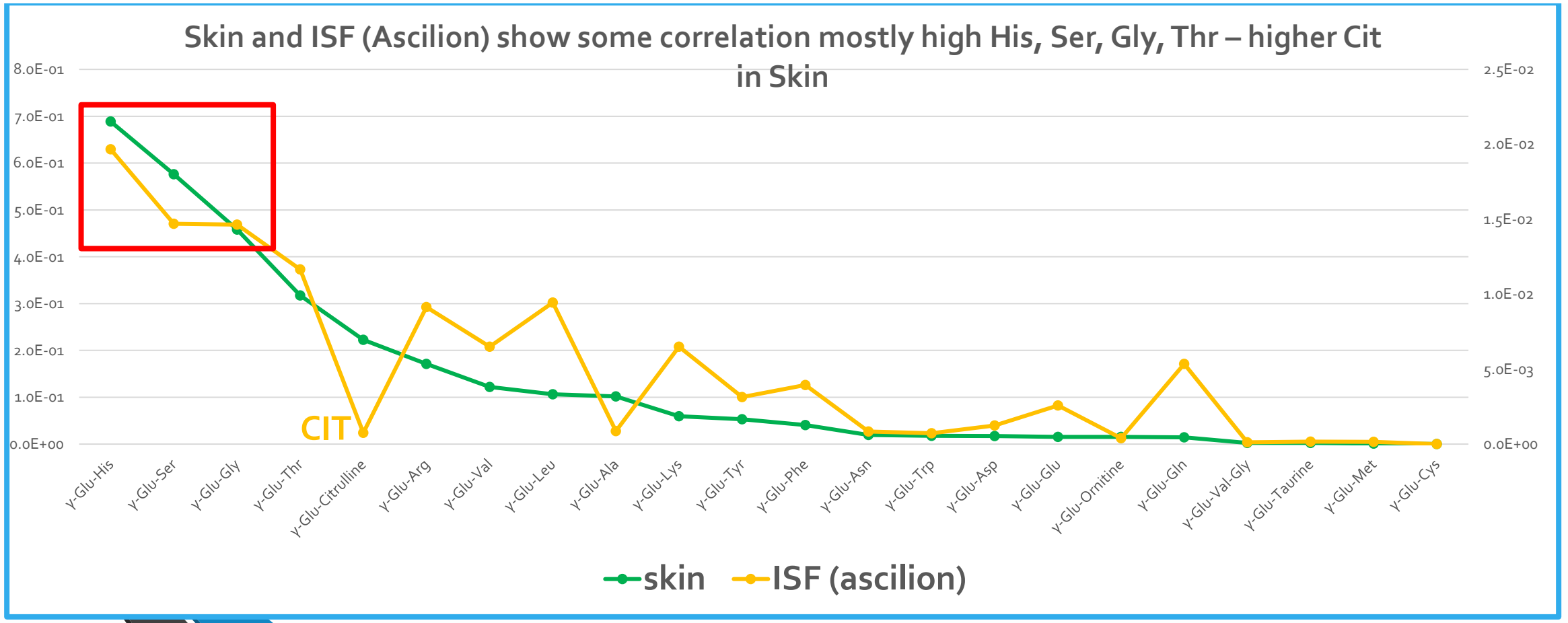


22 Gamma Glu peptides measured across all 4 compartments

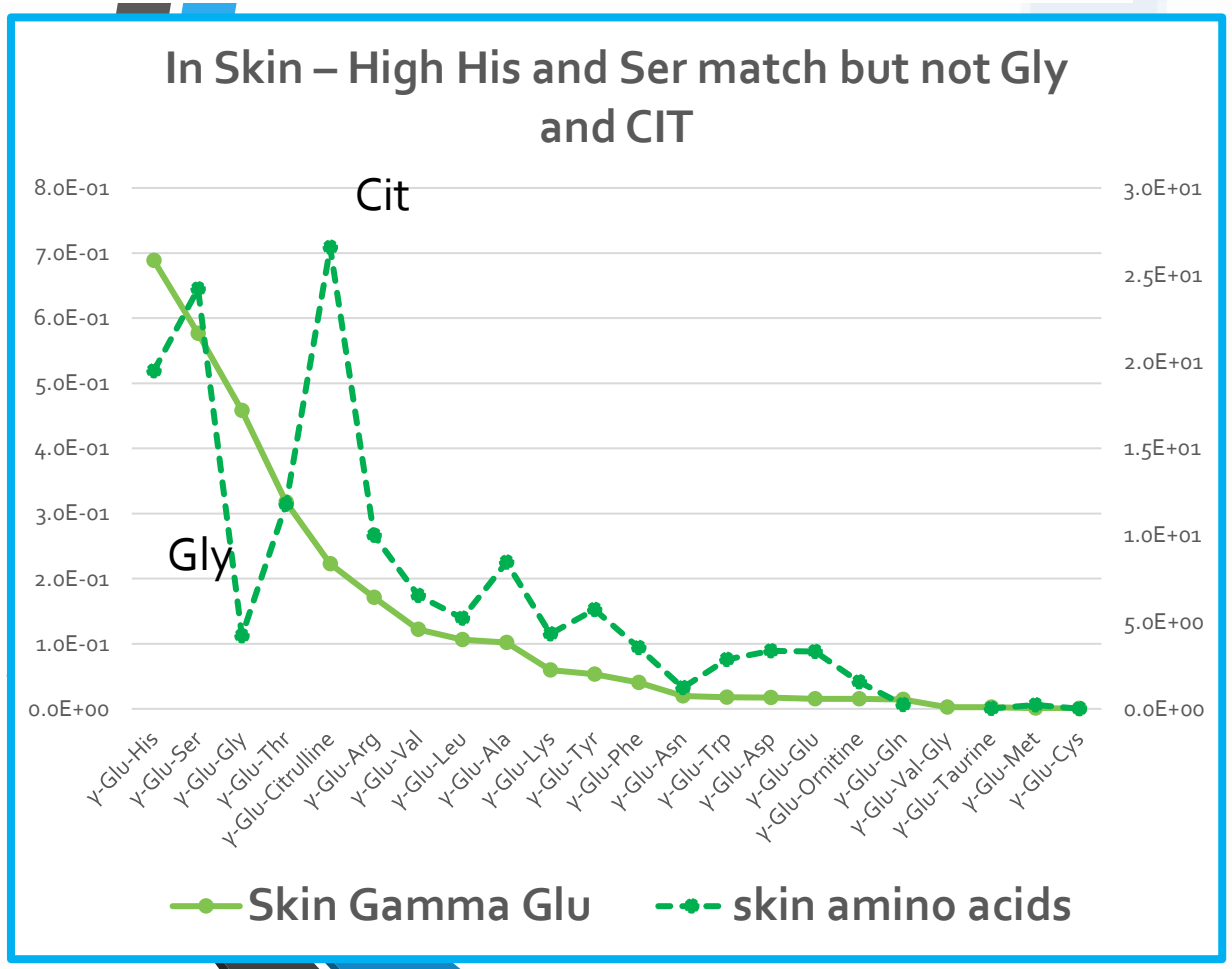
Sorted in Skin

	skin	ISF (Ascilion)	plasma (Virtual Human)	Distal small intestine (Nimble)
γ-Glu-His	6.9E-01	2.0E-02	3.7E-04	1.46E-04
γ-Glu-Ser	5.8E-01	1.5E-02	1.1E-03	2.95E-03
γ-Glu-Gly	4.6E-01	1.5E-02	1.5E-03	1.25E-03
γ-Glu-Thr	3.2E-01	1.2E-02	7.4E-04	3.57E-04
γ-Glu-Citrulline	2.2E-01	7.6E-04	4.1E-04	0
γ-Glu-Arg	1.7E-01	9.1E-03	2.1E-03	6.81E-04
γ-Glu-Val	1.2E-01	6.5E-03	1.1E-03	5.47E-03
γ-Glu-Leu	1.1E-01	9.4E-03	1.5E-03	2.28E-03
γ-Glu-Ala	1.0E-01	8.6E-04	1.4E-03	7.76E-04
γ-Glu-Lys	6.0E-02	6.5E-03	2.6E-03	8.65E-04
γ-Glu-Tyr	5.3E-02	3.1E-03	4.9E-04	6.49E-04
γ-Glu-Phe	4.1E-02	3.9E-03	4.6E-04	8.52E-04
γ-Glu-Asn	2.0E-02	8.4E-04	3.8E-04	4.63E-04
γ-Glu-Trp	1.8E-02	7.2E-04	1.2E-04	1.31E-04
γ-Glu-Asp	1.7E-02	1.2E-03	1.6E-04	3.50E-03
γ-Glu-Glu	1.5E-02	2.6E-03	5.2E-04	1.49E-03
γ-Glu-Ornithine	1.5E-02	3.9E-04	3.4E-04	3.66E-03
γ-Glu-Gln	1.4E-02	5.4E-03	1.5E-02	2.88E-03
γ-Glu-Val-Gly	2.6E-03	1.3E-04	9.6E-05	4.70E-04
γ-Glu-Taurine	2.5E-03	1.6E-04	5.7E-05	0
γ-Glu-Met	9.4E-04	1.4E-04	2.1E-04	2.66E-04
γ-Glu-Cys	3.4E-04	0.0E+00	0.0E+00	5.04E-04

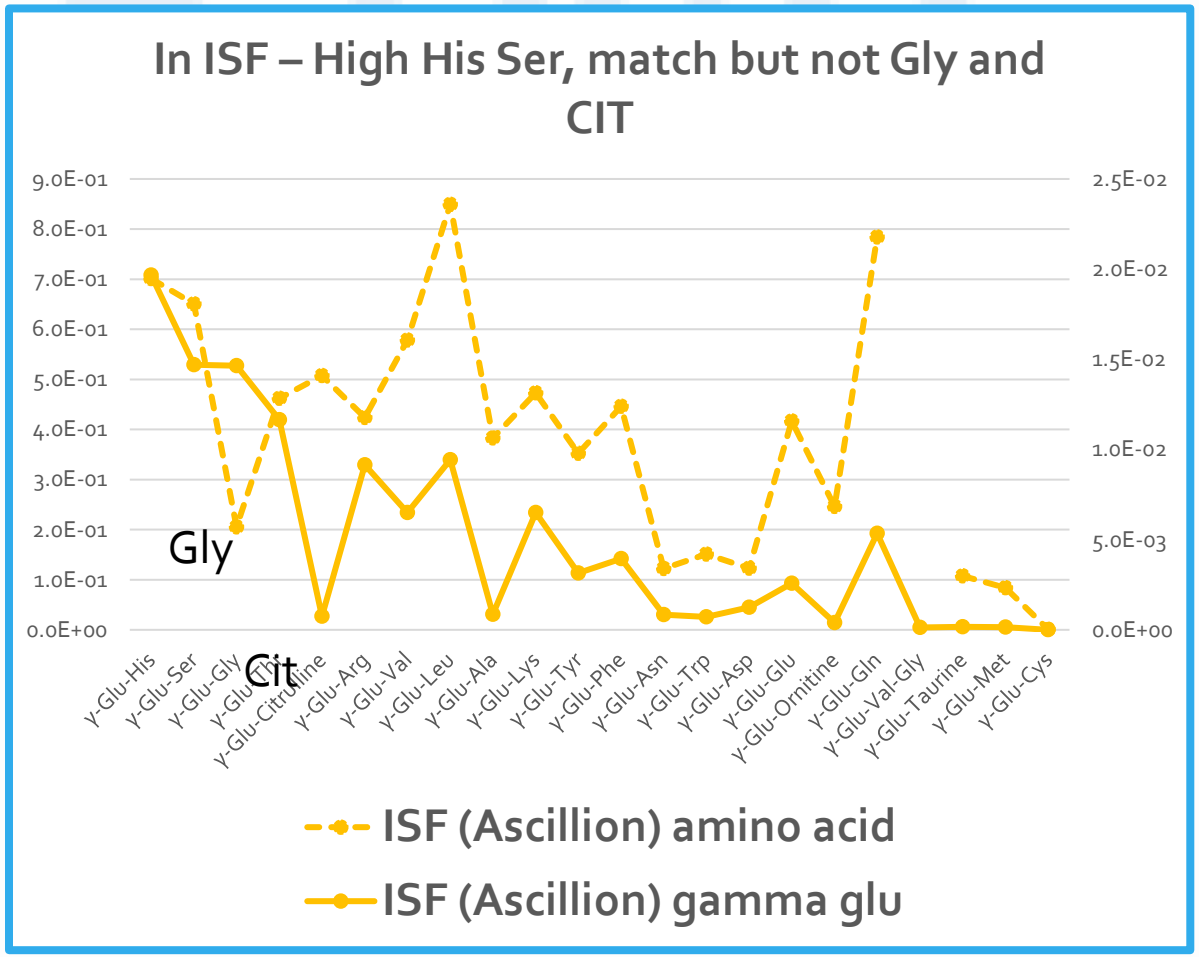
Skin and ISF Correlate well with high GG His Ser Gly; Differ GG-CIT lower in ISF



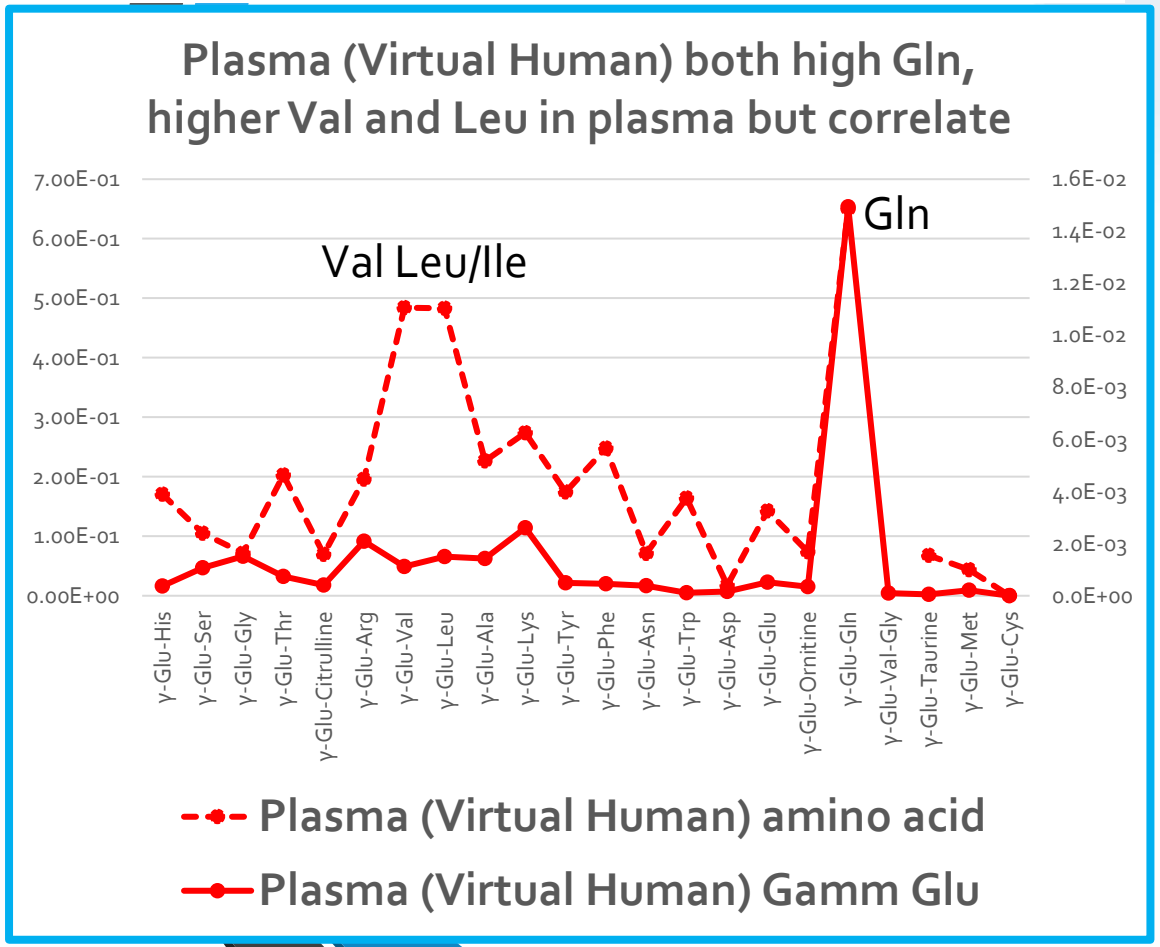
GG and AA in Skin – High His Ser; differ in Gly and CIT



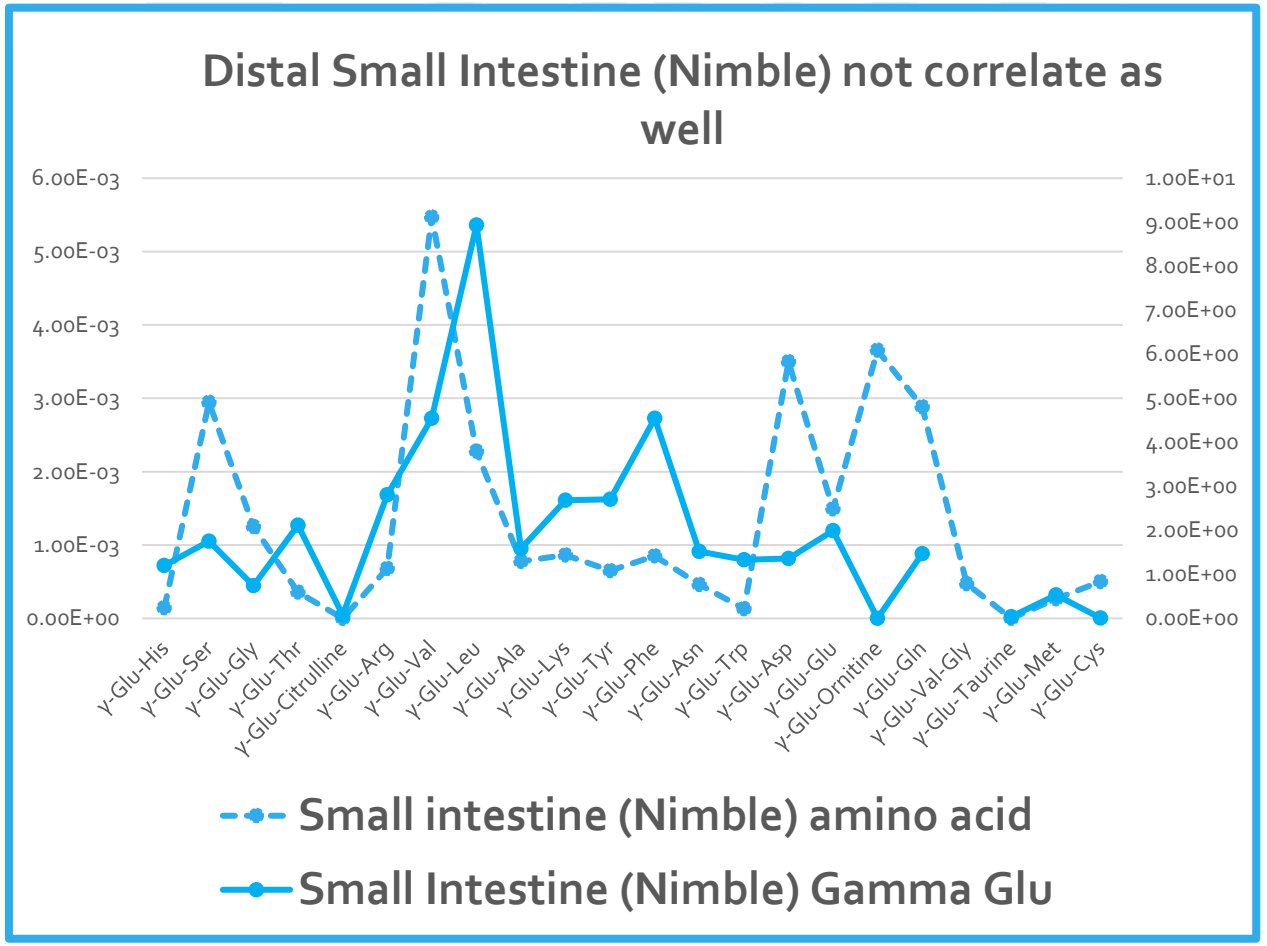
GG and AA in ISF – High His, Ser; Differ in Gly and CIT



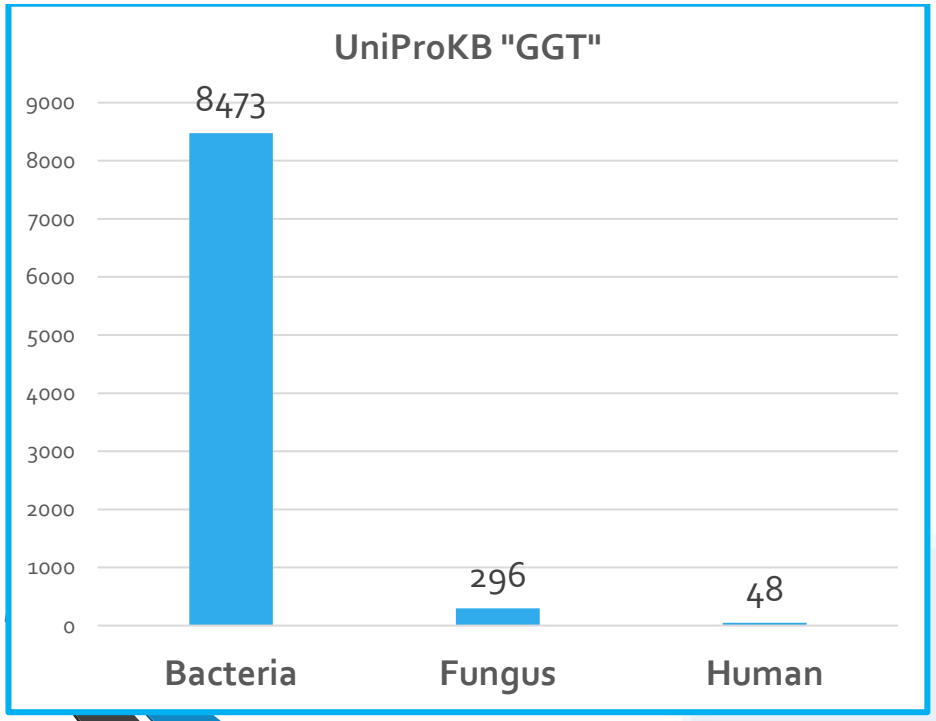
GG and AA in plasma – both high GLN



GG and AA in Intestine are dissimilar



Almost 200x more GGTransferases in bacteria vs human; Does microbiome control GG-peptides?



- Many more transferases in bacteria than human.
- Bacteria control gamma glu dipeptides
- GG peptides often mimic amino acid levels
- Part of amino acid regulation / transport

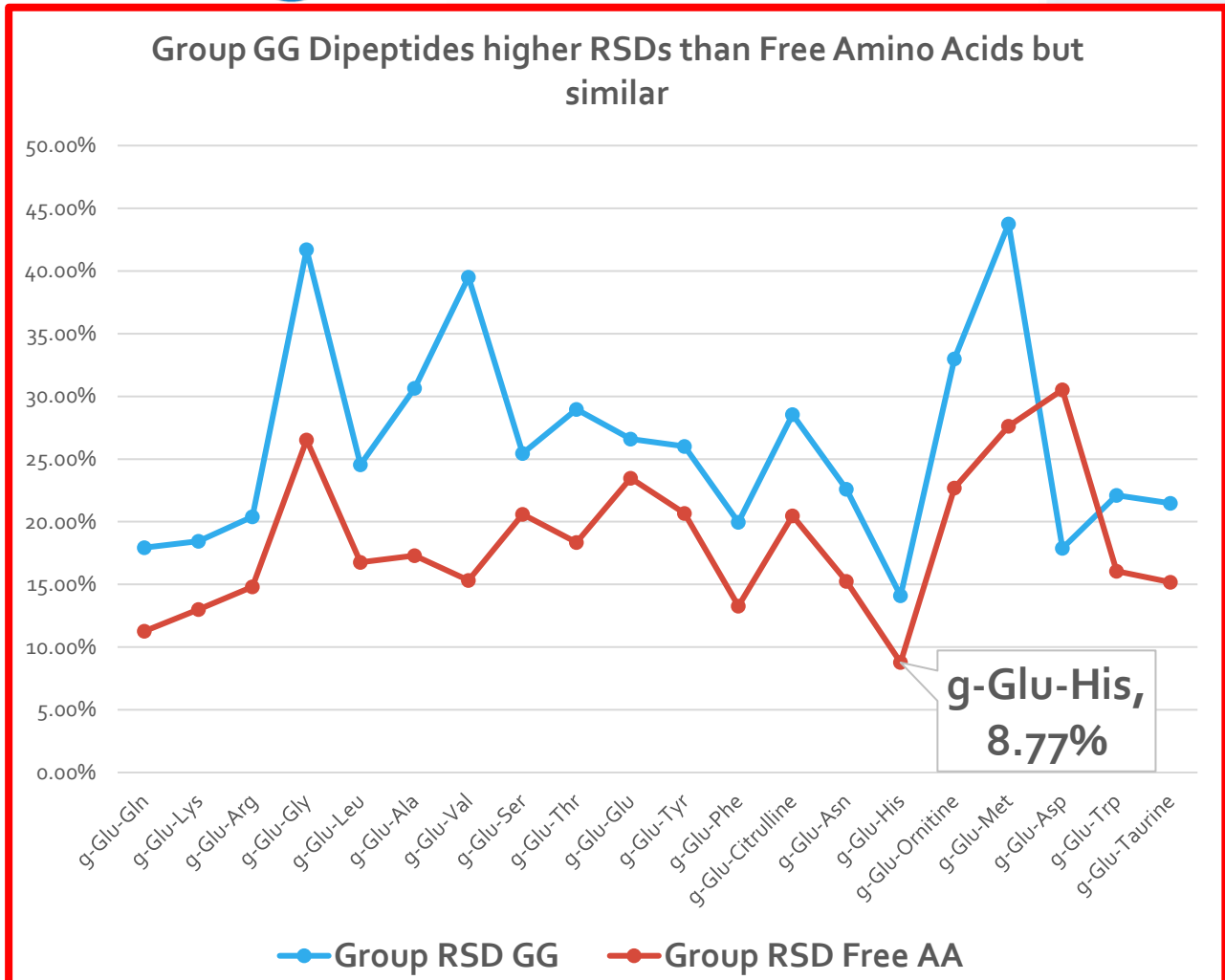
Gamma Glu peptides Summary

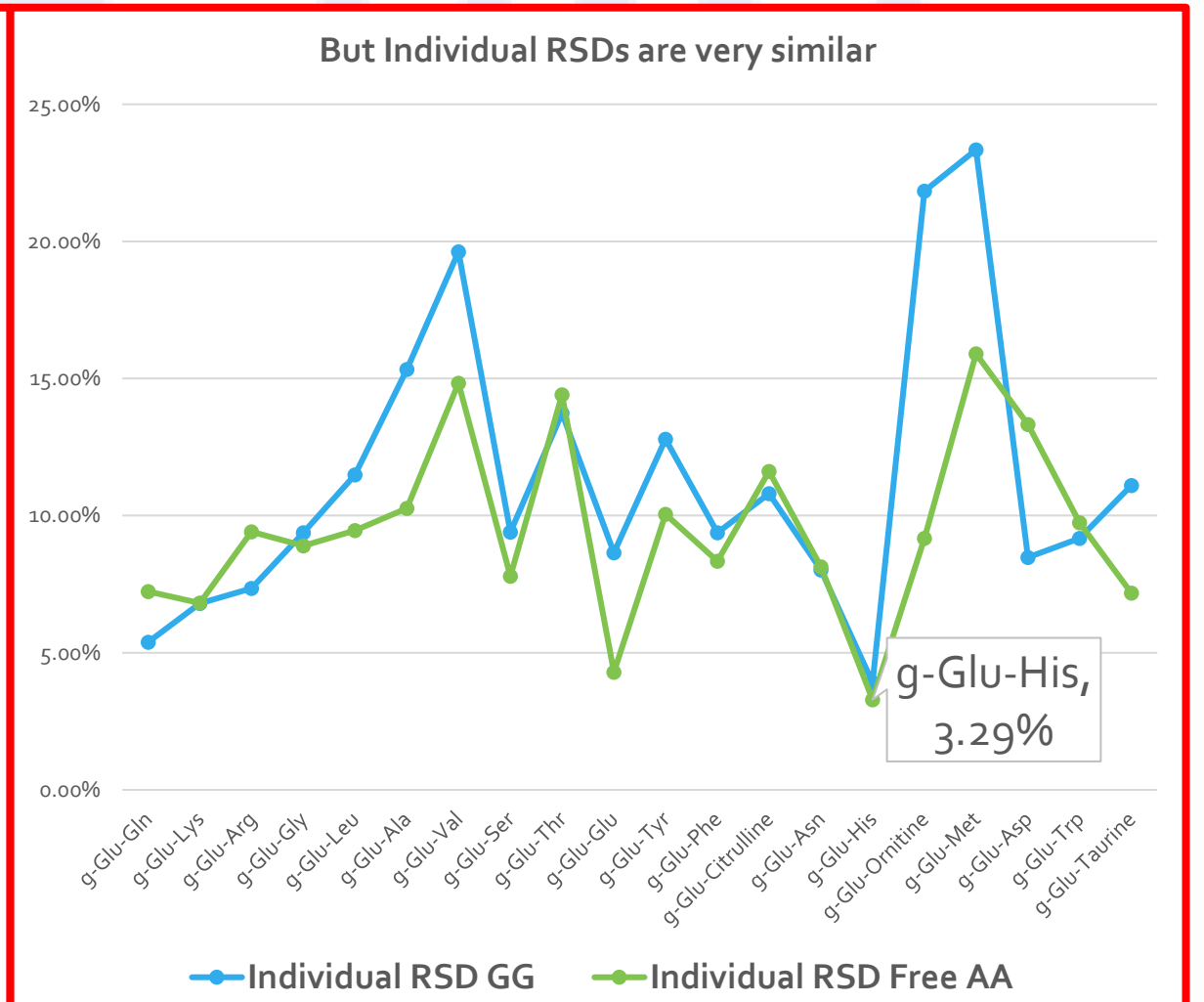
- Skin and ISF correlate well with free amino acids except for higher levels of free CIT and lower free GLY – both share high His Ser.
- Plasma correlates well with their free amino acids with high GLN, except for higher free BCAA – high gamma GLN is not shared by ISF and Skin.
- Intestinal complex – not trending well with other compartments or with free amino acids

There are many references to bacterial gamma glutamyl transferases

- "Bacterial .-glutamyltranspeptidases, physiological function, structure, catalytic mechanism and application" (2022)
- "Bacterial Gamma-Glutamyl Transpeptidase, an Emerging Biocatalyst: Insights Into Structure–Function Relationship and Its Biotechnological Applications" (2021)

RSDs between GG Dipeptides and individual free Amino Acids are very similar





Microbial GG amino acids help control the amino acid pool

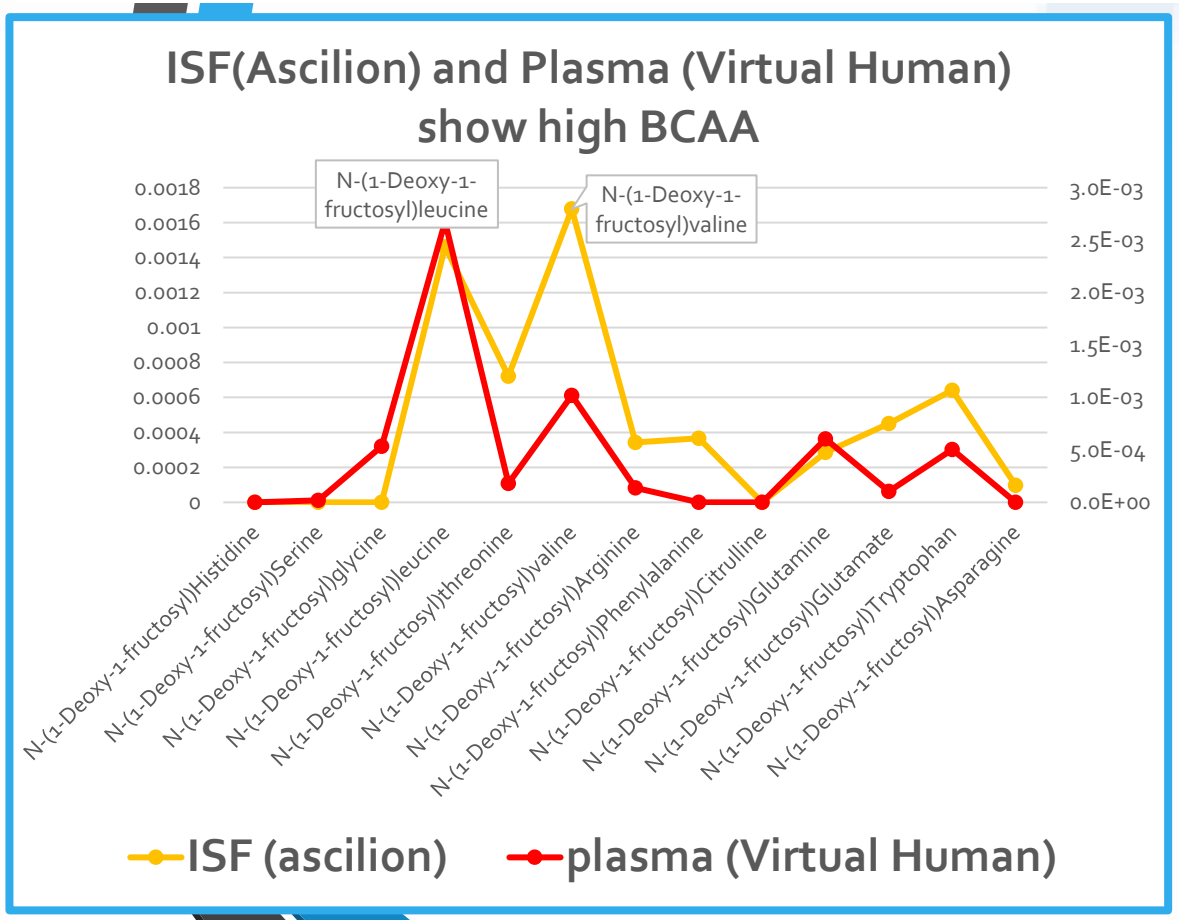
- 1. Skin – microbiota
- 2. Plasma/ISF – bacteria in liver
- 3. Intestinal – microbiota

13 deoxy-fructosyl-amino acids => Maillard Rx?

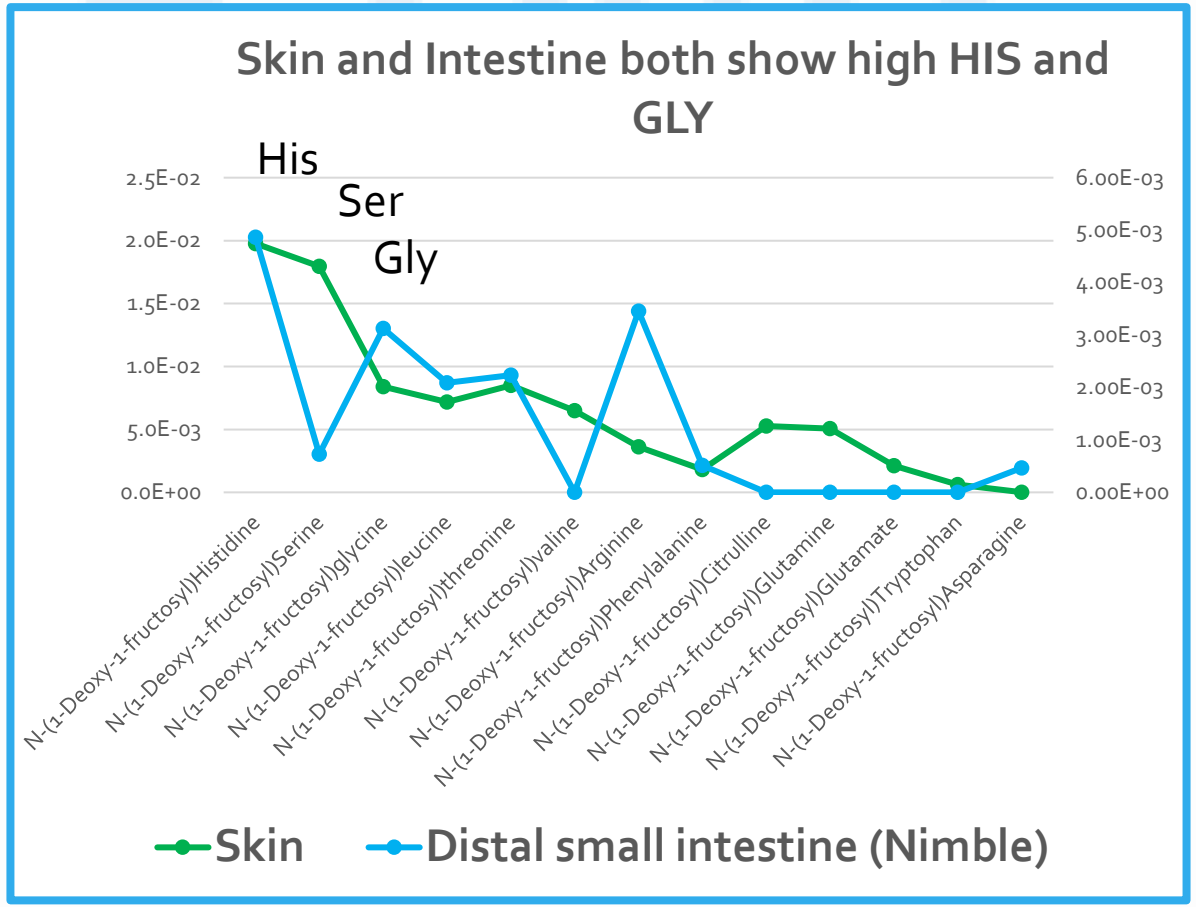
Skin high in His Ser Gly (like amino acids and GG peptides)

	Skin	ISF (Ascillion)	Plasma (Virtual Human)	Distal small intestine (Nimble)
N-(1-Deoxy-1-fructosyl)Histidine	2.0E-02	0	0.0E+00	4.86E-03
N-(1-Deoxy-1-fructosyl)Serine	1.8E-02	0	1.9E-05	7.26E-04
N-(1-Deoxy-1-fructosyl)glycine	8.4E-03	0	5.3E-04	3.13E-03
N-(1-Deoxy-1-fructosyl)leucine	7.2E-03	1.5E-03	2.7E-03	2.09E-03
N-(1-Deoxy-1-fructosyl)threonine	8.5E-03	7.2E-04	1.8E-04	2.23E-03
N-(1-Deoxy-1-fructosyl)valine	6.5E-03	1.7E-03	1.0E-03	0
N-(1-Deoxy-1-fructosyl)Arginine	3.6E-03	3.4E-04	1.4E-04	3.46E-03
N-(1-Deoxy-1-fructosyl)Phenylalanine	1.8E-03	3.7E-04	0.0E+00	5.14E-04
N-(1-Deoxy-1-fructosyl)Citrulline	5.3E-03	0	0	0
N-(1-Deoxy-1-fructosyl)Glutamine	5.1E-03	2.9E-04	6.1E-04	0.00E+00
N-(1-Deoxy-1-fructosyl)Glutamate	2.1E-03	4.5E-04	1.1E-04	0.00E+00
N-(1-Deoxy-1-fructosyl)Tryptophan	6.1E-04	6.4E-04	5.0E-04	0
N-(1-Deoxy-1-fructosyl)Asparagine	1.4E-05	9.8E-05	0.0E+00	4.68E-04

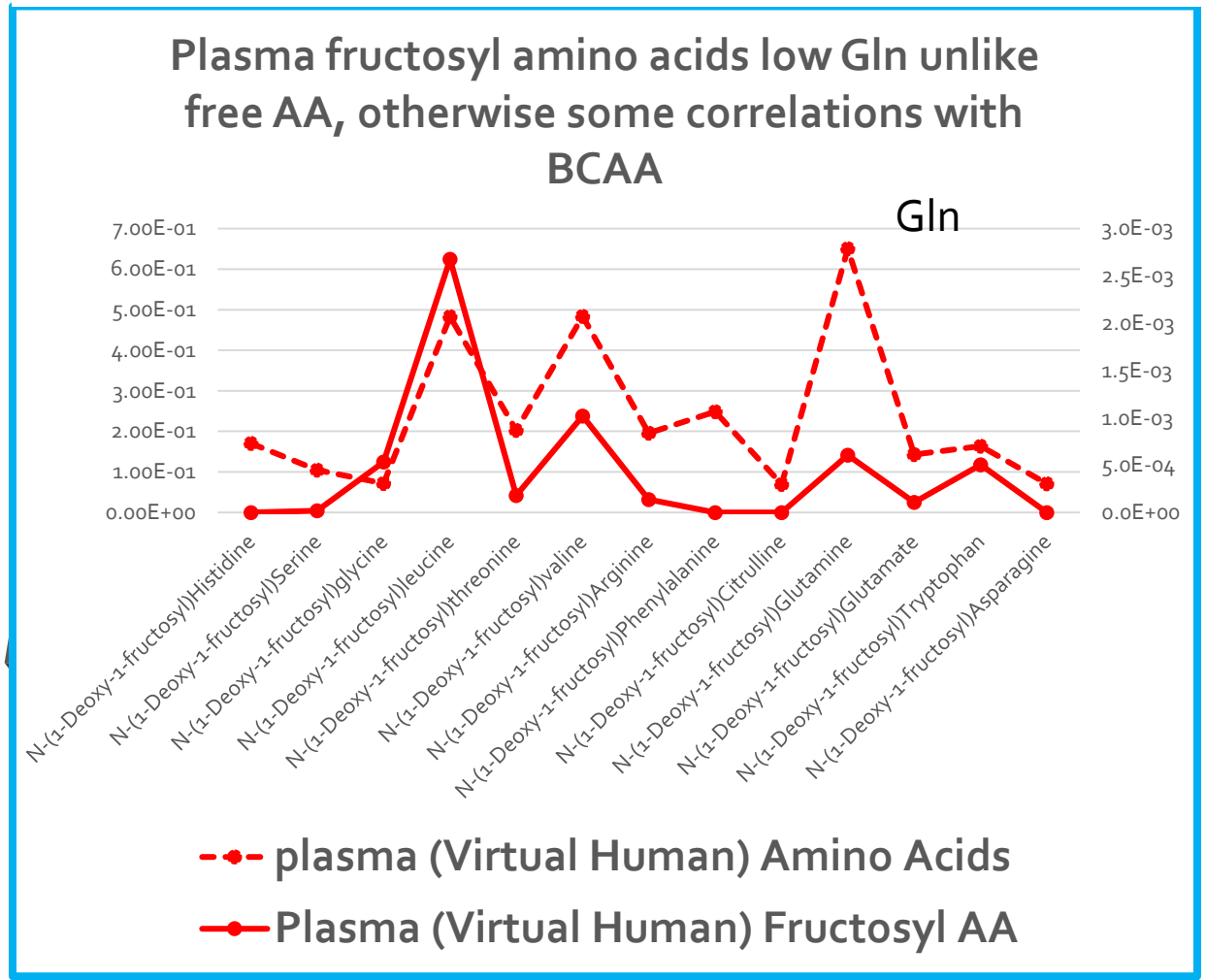
ISF and Plasma FuAA show good correlation with high Leu and Val



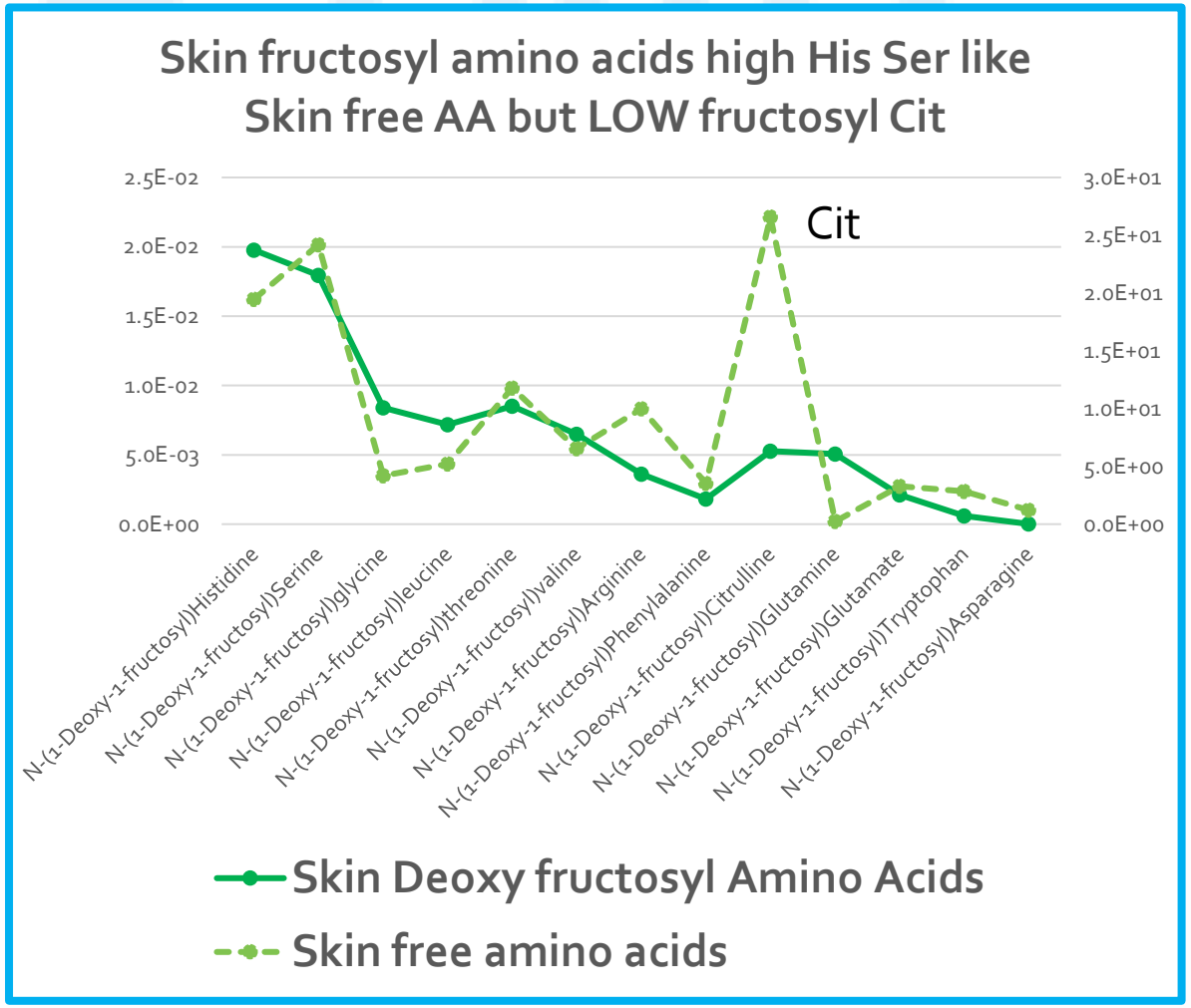
Skin and Intestine FuAA are similar; differ in Ser, Arg



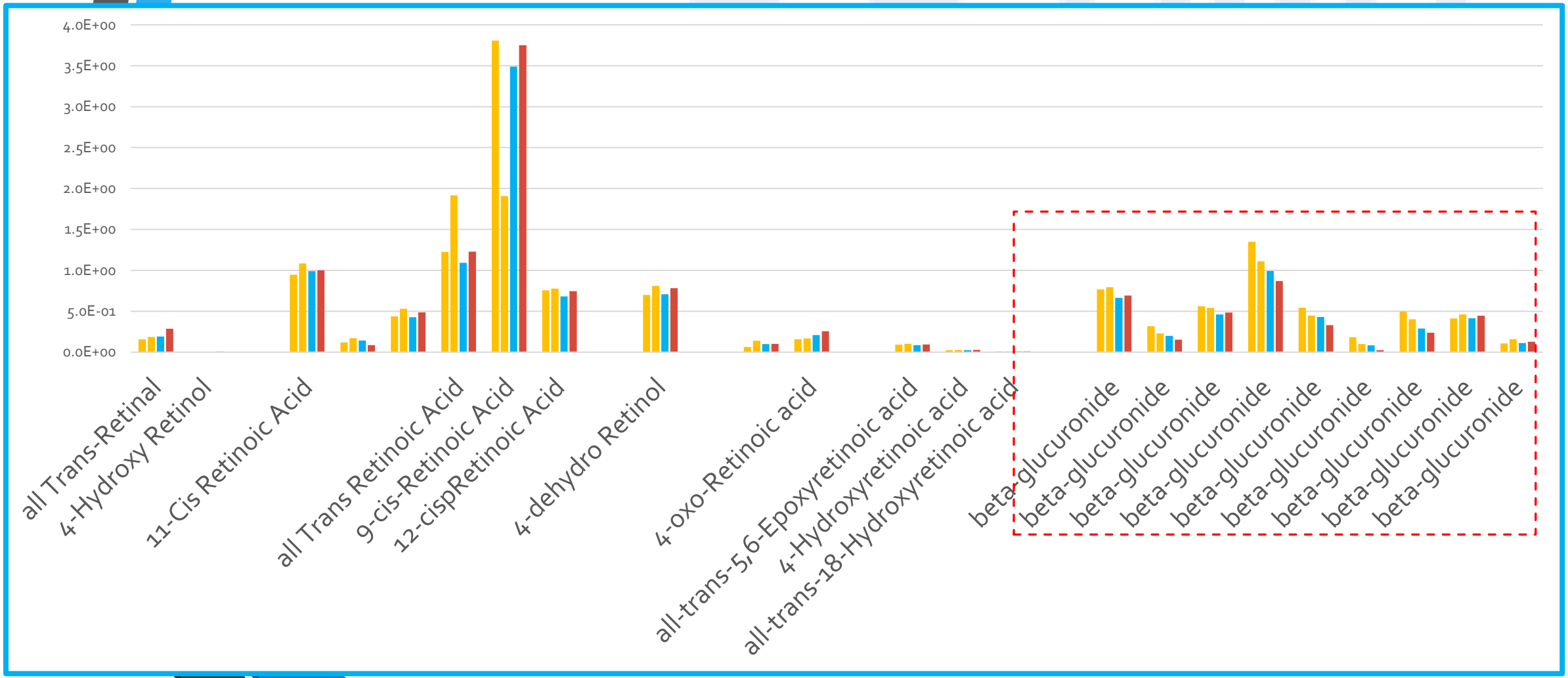
Plasma FuAA match well to plasma free amino acids but for GLN



Skin FuAA match well to Skin free amino acids but for CIT



- Phase 2 metabolism not just in liver



Summary of deoxy-fructosyl-amino acids

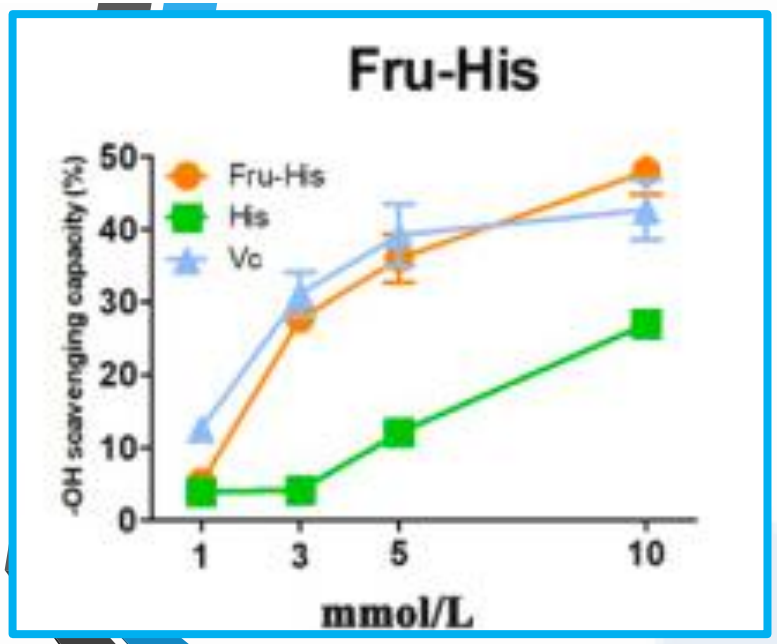
- Skin and Intestinal fluid share high His Gly and low CIT and correlation with free AA
- Interstitial fluid and plasma share high BCAA and low GLN and correlate with free AA

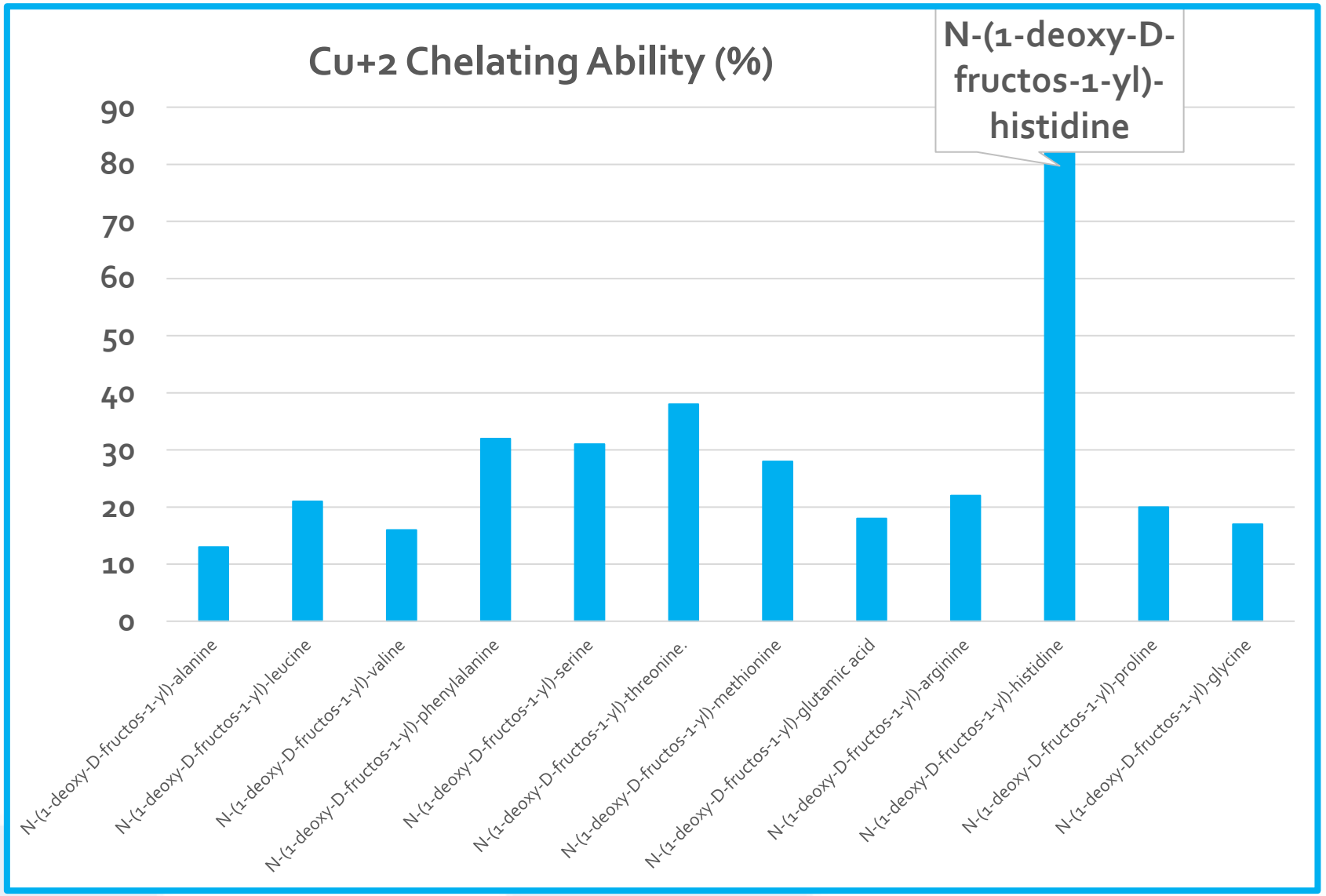
Literature supports AGEs in skin bacteria

- 1. "Evidence of non-enzymatic Glycosylation in E. Coli", (2000)
- 2. "AGEs Secreted by Bacteria are Involved in the Inflammatory Response" (2011)
- 3. "AGE Products in the Skin" (2022)
- 4. "Amadori Products: The Natural Transition Metal (Cu+2, Fe+2, Zn+2) Chelators" (2024)

HMT

Deoxyy Fructosyl Histidine has hydroxyl radical scavenging capacity equal to Vit C





Suggest two sources of fructosyl amino acids – all bacterial

1. Epithelial cells/ skin cells serving skin and small intestinal fluids

2. Liver

Certain bacteria have evolved specialized enzymatic systems to "deglycate" these compounds.

Some bacteria capable of degrading these products may be considered beneficial or "probiotic" by releasing essential amino acids

13 Glycines conjugates – Phase 2 metabolites?

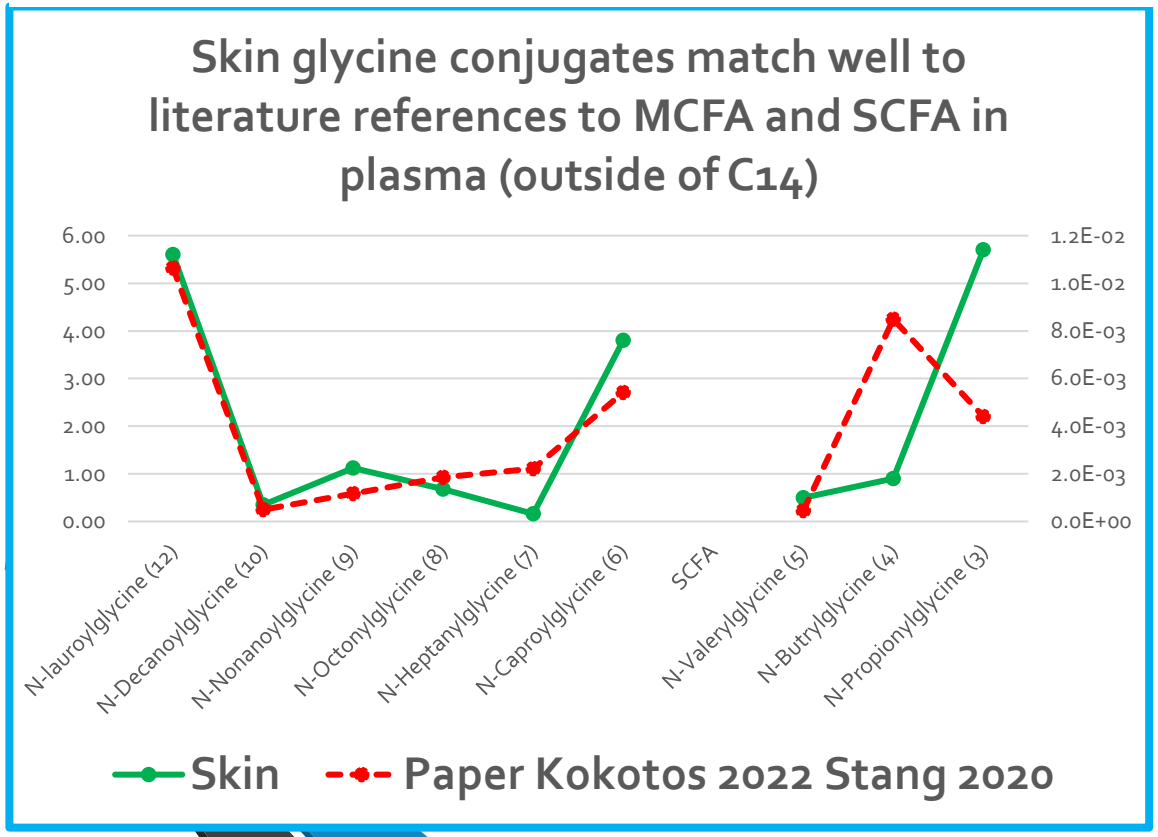
MCFA	Skin	ISF (Ascilion)	plasma (Virtual Human)	Distal small intestine (Nimble)
17	5.5E-01	0	0	0
16	8.8E-01	0	0	0
N-Myristoylglycine (14)	9.7E+01	0	0	0
13	1.9E+00	0	0	0
N-lauroylglycine (12)	2.7E-01	0	0	0
N-Decanoylglycine (10)	2.4E-03	0	2.5E-04	0
N-Nonanoylglycine (9)	1.9E-03	0.0E+00	0	0
N-Octonylglycine (8)	1.8E-01	0.0E+00	6.5E-05	0
N-Heptanylglycine (7)	1.9E-01	2.5E-05	2.0E-04	1.55E-04
N-Caproylglycine (6)	1.5E-04	5.8E-05	6.6E-05	1.87E-04
SCFA				
N-Valerylglycine (5)	4.6E-04	4.2E-05	1.3E-05	0
N-Butrylglycine (4)	8.5E-03	0.0E+00	6.3E-05	0
N-Propionylglycine (3)	4.4E-03	0.0E+00	7.3E-04	9.29E-04

Very HIGH C14

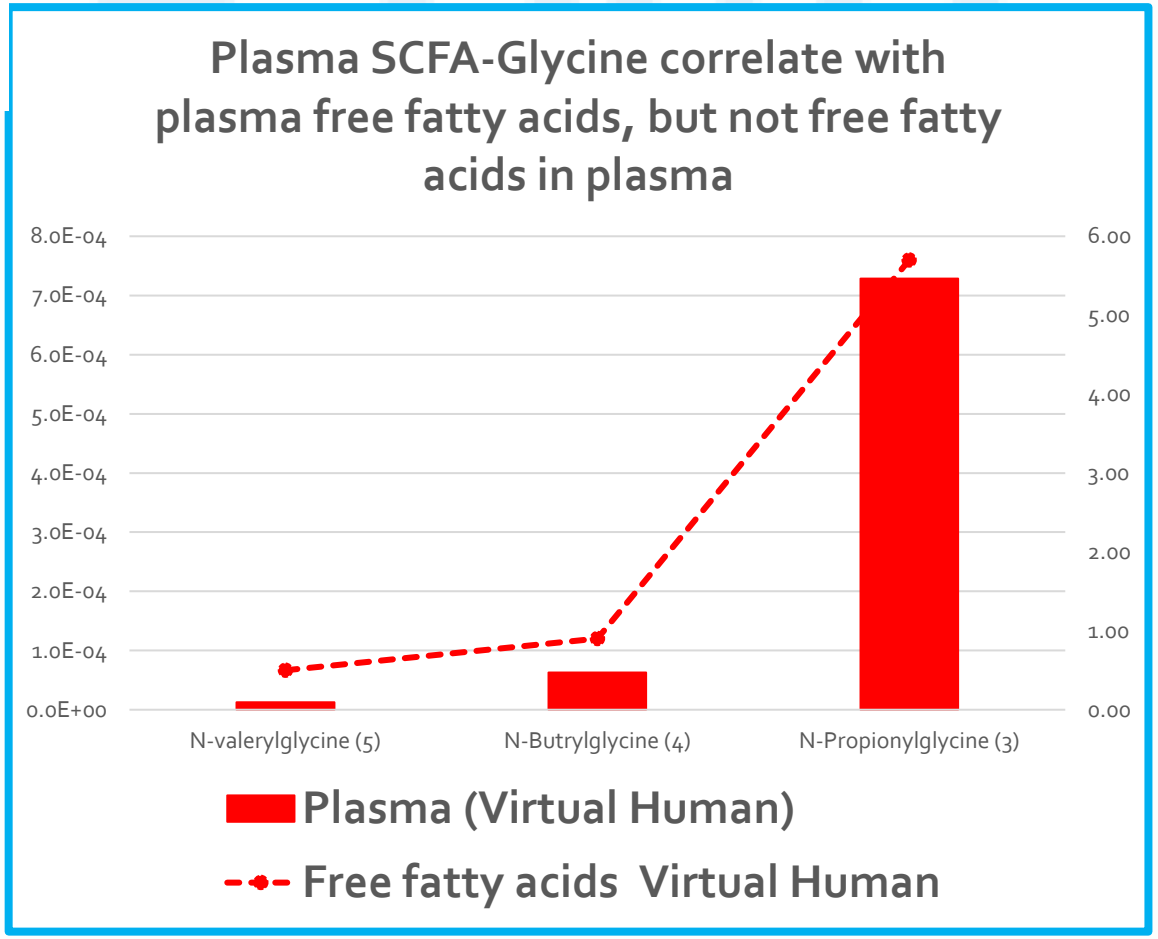
Phase 2?

Related? Different pathways? Related to microbiome?

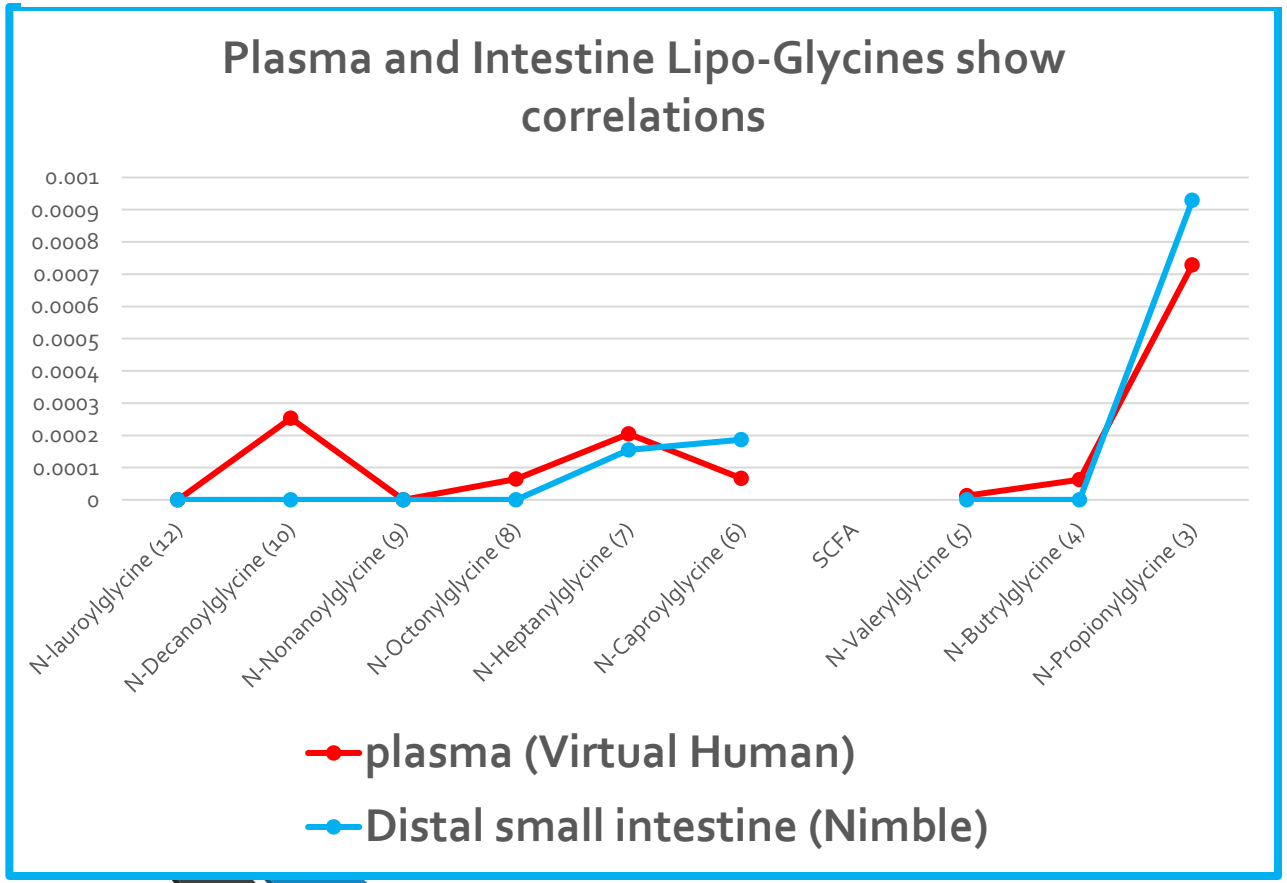
Skin MC/SC glycine conjugates correlate with plasma free fatty acids



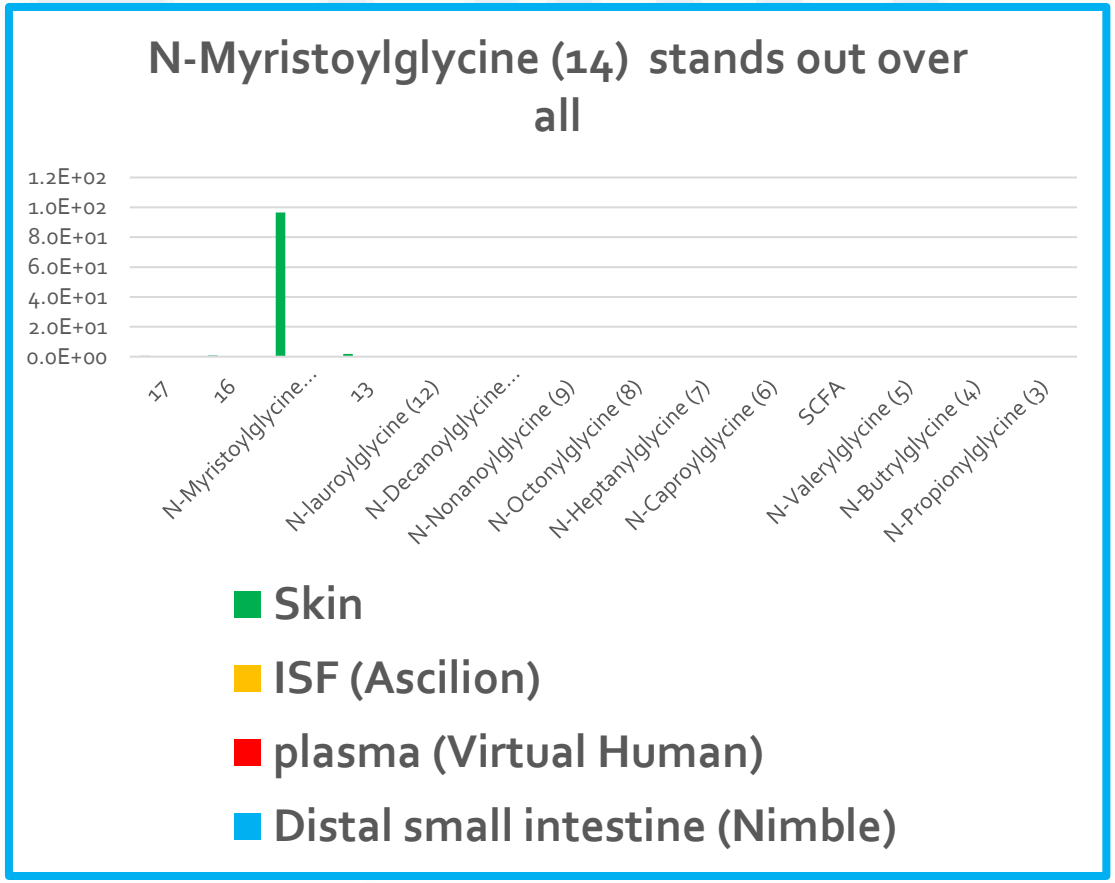
Plasma SC glycine conjugates correlate well to free plasma SCFAs



C12-C6 and SC glycine conjugates in plasma correlate with free AA



Myristoyl-Glycine 100 x all other glycine conjugates and lack correlation



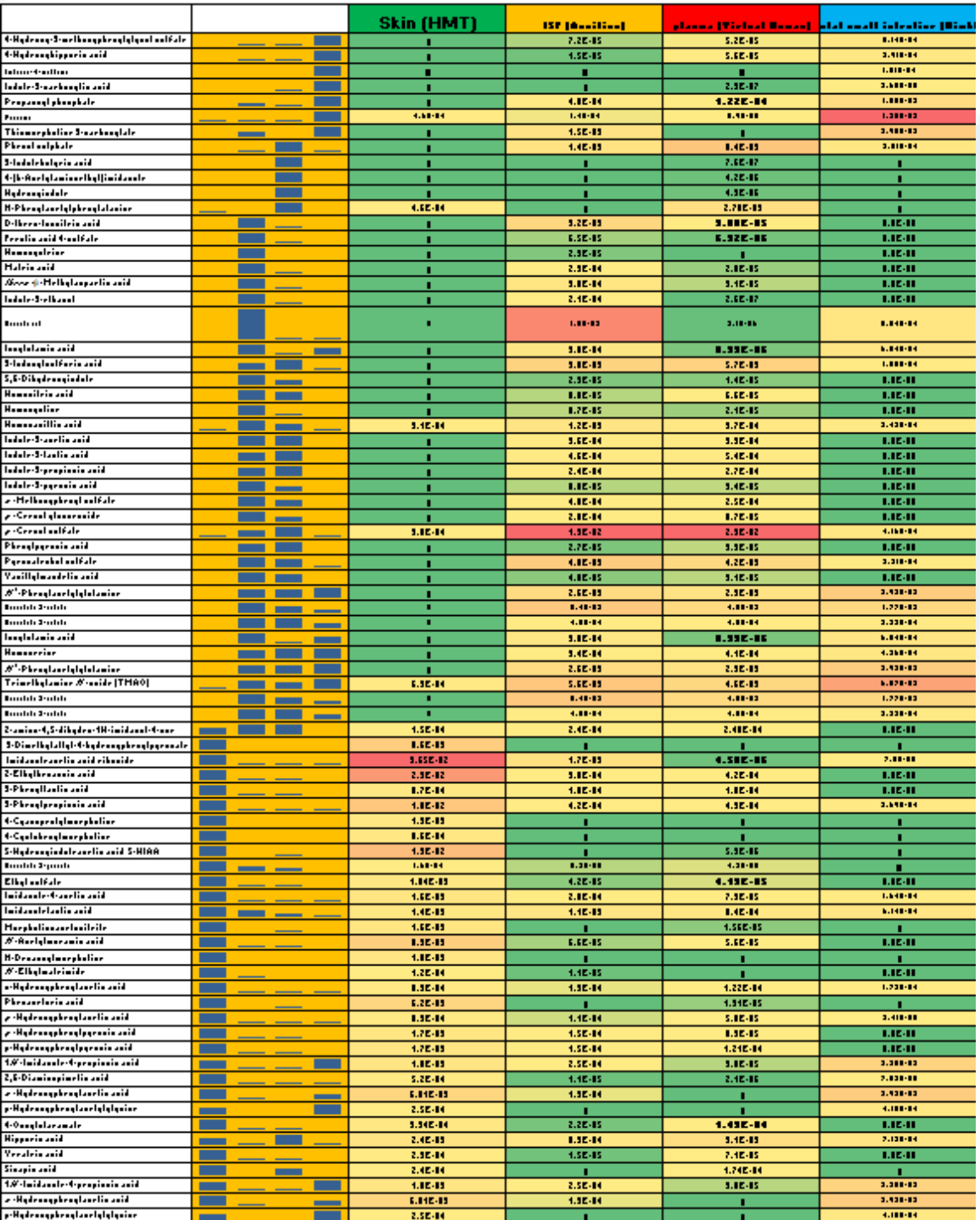
Summaries

C14 -Glycine related to skin N-Myristoyl Transferases

MC-Glycine linked to bacterial secretion system

SC-Glycine linked to bacterial Phase 2 metabolism

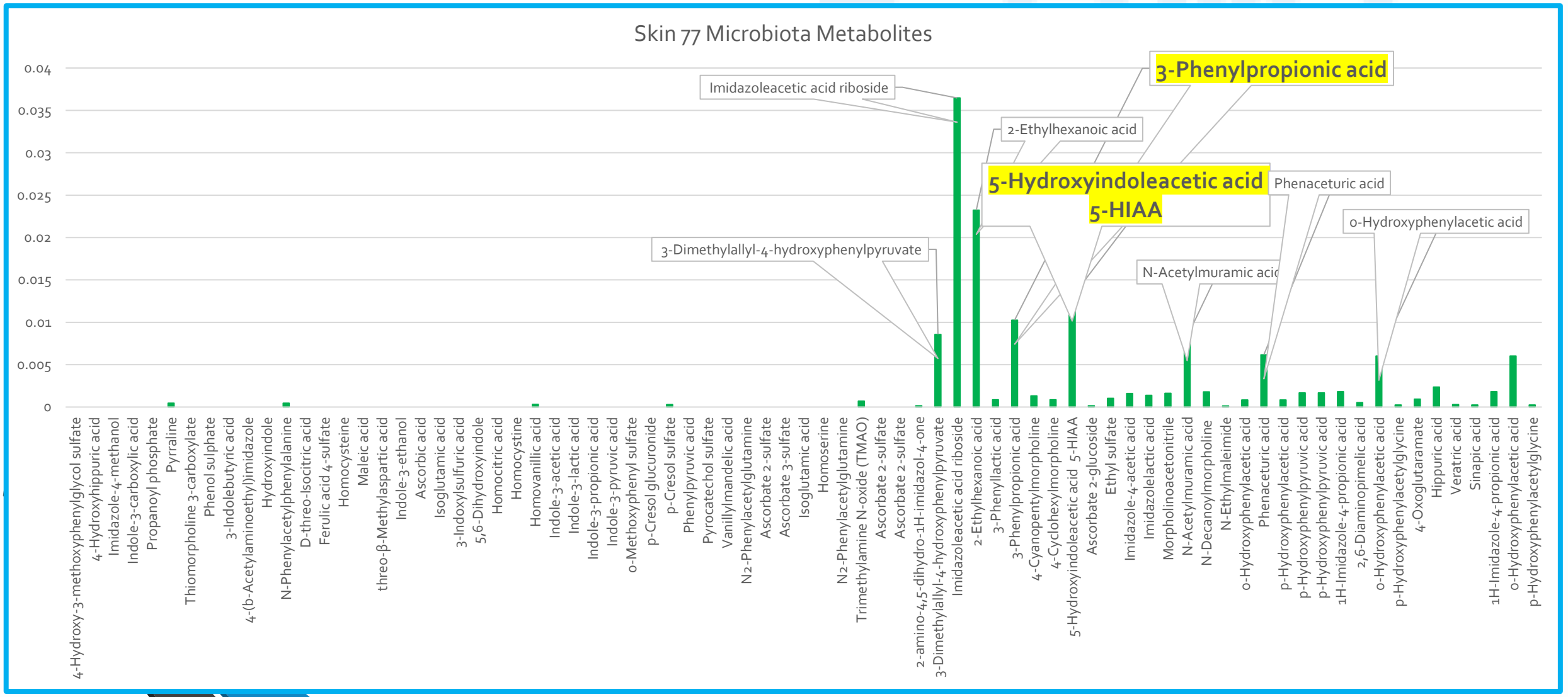
NMT C12-C7 Skin	N-myristoyltransferase (C14) (NMT) myristoylation regulates protein function and localization. In skin cells, NMT1 and NMT2 are expressed and implicated in various skin cell functions.
Bacterial C6-C10 Plasma Intestine	Christopher Icke et. al, (2021) Glycine acylation and trafficking of a new class of bacterial lipoprotein by a composite secretion system eLife 10:e63762.
Phase 2 C2-5 Plasma Intestine	Phase 2 Glycine conjugation converts organic acids, into inactive, water-soluble forms. Phase 2 can occur in liver, kidneys, adipose, lung, skin and Intestines.



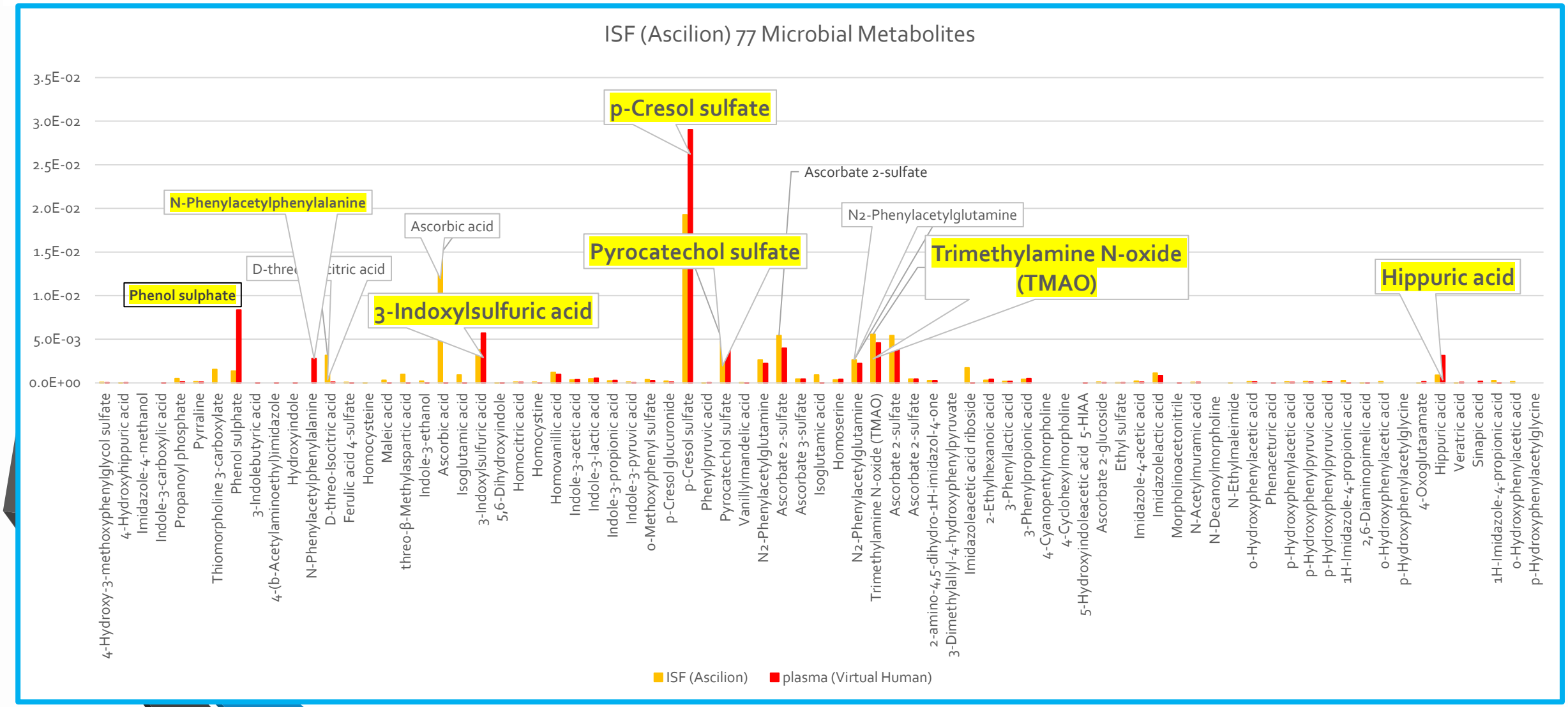
77 “microbial” metabolites

Distributed across all 4 compartments

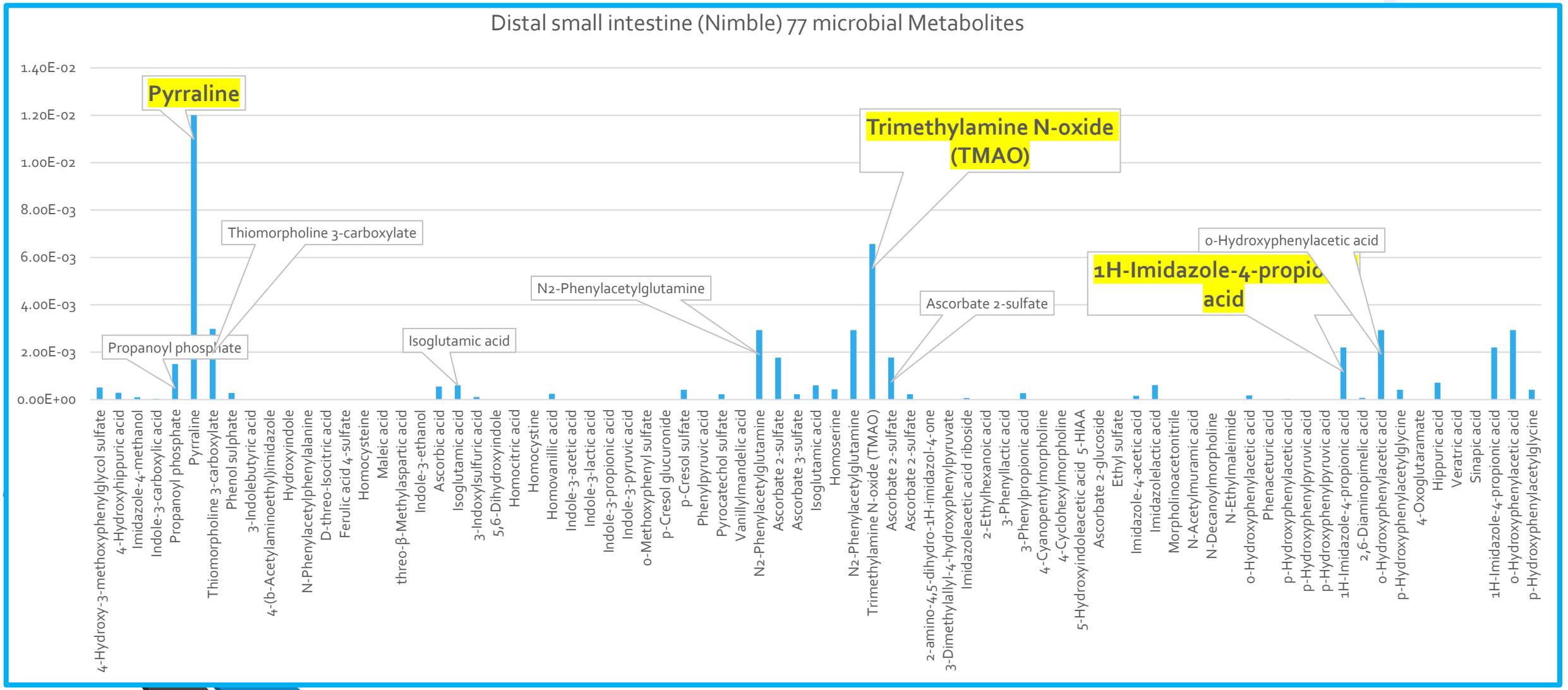
~ 30 Metabolites stand out in Skin



~ 25 Metabolites stand out and common to ISF and Plasma



Several Metabolites stand out in Small Intestine



Suggest 3 microbiomes

Skin	Imidazoleacetic acid riboside (IAA-R)	Bacterial	His	Precursor to IAA		moderate
	2-Ethylhexanoic acid	Gut derived				moderate
	3-Phenylpropionic acid	Gut derived	Phe			Good metabolite
	N-Acetylmuramic acid	Bacterial		Bacterial cell wall		moderate
	5-Hydroxyindoleacetic acid 5-HIAA	Bacterial	Trp			Marker of SIBO
	3-Dimethylallyl-4-hydroxyphenylpyruvate (3DMA-4HPP)	Bacterial				moderate
	o-Hydroxyphenylacetic acid	Gut derived	Phe Tyr			Marker of SIBO
ISF Plasma	Phenol Sulfate	Gut derived phenol	TYR PHE	Tyrosine phenol-lysase	Sulfate in liver	moderate levels
	Threo isocitrate	Bacterial	TCA cycle	isocitrate dehydrogenase		moderate levels
	3-indoxysulfate	Gut derived	TRP			marker of dysbiosis
	cresol sulfate	Gut derived	TYR PHE	Fermentation		uremic toxin
	phenylacetylglutamine	Gut derived phenylacetic acid	PHE		GLN in liver	uremic toxin
	pyrocatechol sulfate	Gut derived phenol	TYR PHE			Complex (Diet, microbiome)
	phenylacetylphenylalanine	Gut derived phenylacetic acid	PHE			moderate levels
	Hippuric acid	Gut derived benzoic acid	polyphenols		Gly in liver	Marker of SIBO
	TMAO	Gut derived TMA			Oxide in liver	Generally good
Small Intestine	Pyrraline (2-Formyl-5-(hydroxymethyl)pyrrole-1-norleucine)	AGE - bacterial				moderate
	1*H*-Imidazole-4-propionic acid	Gut derived from Urocanate	His			moderate
	o-Hydroxyphenylacetic acid	Gut derived	Phe Tyr			Marker of SIBO
	Isoglutamic acid	Bacterial				
	N^2-Phenylacetylglutamine	Gut derived phenylacetic acid	PHE		GLN in liver	uremic toxin
	Trimethylamine *N*-oxide (TMAO)	Gut derived TMA			Oxide in liver	Generally good
	Ascorbate 2-sulfate	Phase 2 metabolite				

Epidermis exhibits peptide rich anti oxidants

			Skin (HMT)	ISF (Ascilion)	plasma (Virtual Human)	Distal small intestine (Nimble)
epidermis	Ala-His (fillaggrin)	His peptide	3.9E-01	1.0E-03	2.77E-06	2.63E-05
epidermis	Homoanserine (GABA-meHIS) brain	His peptide	1.3E-01	4.3E-04	0	3.19E-02
epidermis	Cys-Ala-His (Fillaggrin)	His peptide	1.6E-02	0	0	0
epidermis	Gly-His-Gly (Fillaggrin)	His peptide	1.3E-02	1.7E-04	7.53E-07	3.07E-03
epidermis	Phe-Leu (Fillaggrin)	Phe peptide	1.2E-02	1.7E-04	8.28E-05	4.15E-05
epidermis	Gly His Ser (Fillaggrin)	His peptide	8.1E-03	0	0	0
epidermis	Set Thr His (Fillaggrin)	His peptide	5.7E-03	0	0	0
epidermis	Ala Gly Tyr	Tyr peptide	3.7E-03	4.1E-04	0	1.07E-03
epidermis	*N*-Acetylcarnosine	*His peptide*	3.0E-03	1.0E-03	5.33E-04	0.0E+00
epidermis	γ-Glu-Val-Gly		2.6E-03	1.3E-04	2.12E-06	4.70E-04
epidermis	Ophthalmic acid (γ-Glu-2ABA-Gly)	GSH regulation	1.7E-03	4.8E-04	1.63E-04	4.36E-03
epidermis	Phe Phe	Phe peptide	1.4E-03	4.0E-05	1.78E-05	0.0E+00
epidermis	Ala-His-Gly (fillaggrin)	His peptide	9.4E-04	2.3E-04	3.28E-06	6.57E-04
epidermis	Cystathionine brain	Cysteine synthesis	5.6E-04	4.3E-04	6.73E-04	4.61E-04
epidermis	Carnosine (b-ala-His) muscle	His peptide	2.1E-04	1.4E-05	2.72E-05	0.0E+00
epidermis	Anserine (b-Ala-MeHis) muscle	His peptide	1.9E-04	9.7E-05	7.01E-04	7.31E-04

The major anti oxidants in the epidermis are dominated by His containing peptides and other peptides and distinctively different than ISF, Plasma and small intestine.

Filaggrin

MSTLLENIFAIINLFKQYSKKDKNTDTLSKKELKELLEKEFRQILKNPDDPDMVDVFMDHLDIDHNKK
IDFTEFLLMVFKLAQAYYESTRKENLPISGHKHRKHSHHDKHEDNKQEENKENRKRPSSLERRNNR
KGNKGRSKSPRETGGKRHESSSEKKERKGYSPTHREEEYGKNHHNSSKKEKNKTENTRLGDNRKR
LSERLEEKEDNEEGVYDYENTGRMTQKWIQSGHIATYYTIQDEAYDTTDSLLEENKIYERSRSSDGK
SSSQVNRSRHENTSQ /EQS
RDGSRHPRSHDEDRA QS
ADSSRHSATGRGQAS QEST
RGRSGERSGRSGSSL TIR
GHPGSSRGGRQGSH QSG
DGTRHSGSRHHEASS VSG
SASRNHHGSAQEQSF QAR
SSAGERHGSRHQLQS SVS
GHGQAGHHQQSHQI EQA
RDNSRHSASQDGQD GQS
GSRSASRTTRNEEQSF QDS
DSEGHSEDSERWSGS AET
SSGGQAASSHEQARS SDS
EGHSEESDTQSVSGH TRTS
TGRRQGSHHEQARDS HTT
SQGRSDASHGQSGSI GS
RTSRHQGSSVSQDSD GHS
ADSSRQSGTHHTESS RDS
GHRGSSGSQVTNSEC /SSH
EQSESTHGQTAPSTG SVD
RSGHSGYHHSHTTPQ SSR
HSQVGQGESAGSKTS IPRS
HQEDRASHGHSAESS GTG
RRQDSSVVGDSGNR RSG
RSGSFLYQVSTHEQSE GGR
QGSHYEQSVDSSGHSGSHHSHTTSQERSDVSRGQSGSRSVSRQTRNEKQSGDGSRHSGSRHH
EASSRADSSRHSQVGQGQSSGPRTSRNQGSSVSQDSDSQGHSEDSERWSGSASRNHLGSAWE
QSRDGSRHPGSHHEDRAGHGHSADSSRQSGTRHTESSSRGQAASSHEQARSSAGERHGSHHQ
LQSADSSRHSGIGHGQASSAVRDSGHRGYSGSQASDSEGHSEDSDTQSVSAQGKAGPHQQSH
KESARGQSGESSGRSGSFLYQVSTHEQSESTHGQSAPSTGGRQGSHYDQAQDSSRHSASQEGQ
DTIRGHPGPSRGGRQGSHQEQSVDRSGHSGSHHSHTTSQGRSDASRGQSGSRSASRKTYDKEQ

Filaggrin sequence

Filaggrin is abundant and essential in healthy human skin, playing a critical role in forming a strong skin barrier

SGDGSRHSGSHHHEASSWADSSRHSLVGQGQSSGPRTSRPRGSSVSQDSDSEGHSEDSERRS
GSASRNHHGSAQEQSRDGSRHPRSHHEDRAGHGHSAESSRQSGTHHAENSSGGQAASSHEQ
ARSSAGERHGSHHQQSADSSRHSGIGHGQASSAVRDSGHRGSSGSQASDSEGHSEDSDTQSV
SAHGQAGPHQQSHQESTRGRSAGRSGRSGSFLYQVSTHEQSESAHGRTGTSTGGRQGSHHKQA
RDSSRHSTSQEGQDTIHGHPGSSSGGRQGSHYEQLVDRSGHSGSHHSHTTSQGRSDASHGHS
GSRSASRQTRNDEQSGDGSRHSGSRHHEASSRADSSGHSQVGQGQSEGPRTSRNWGSSFSQ
DSDSQGHSEDSERWSGSASRNHHGSAQEQLRDGSRHPRSHQEDRAGHGHSADSSRQSGTRH
TQTSSGGQAASSHEQARSSAGERHGSHHQQSADSSRHSGIGHGQASSAVRDSGHRGYSGSQA
SDNEGHSEDSDTQSVSAHGQAGSHQQSHQESARGRSGETSGHSGSFLYQVSTHEQSESSHGW
TGPSTRGRQGSRHEQAQDSSRHSASQDGQDTIRGHPGSSRGGRQGYHHEHSVDSSGHSGSHH
SHTTSQGRSDASRGQSGSRSASRTTRNEEQSGDGSRHSGSRHHEASTHADISRHSQAVQGQSE
GSRRSRRQGSSVSQDSDSEGHSEDSERWSGSASRNHHGSAQEQLRDGSRHPRSHQEDRAGH
GHSADSSRQSGTRHTQTSSGGQAASSHEQARSSAGERHGSHHQQSADSSRHSGIGHGQASSA
VRDSGHRGYSGSQASDNEGHSEDSDTQSVSAHGQAGSHQQSHQESARGRSGETSGHSGSFLY
QVSTHEQSESSHGWTGPSTRGRQGSRHEQAQDSSRHSASQYGQDTIRGHPGSSRGGRQGYHH
EHSVDSSGHSGSHHSHTTSQGRSDASRGQSGSRSASRTTRNEEQSGDSSRHSVSRHHEASTHA
DISRHSQAVQGQSEGSRRSRRQGSSVSQDSDSEGHSEDSERWSGSASRNHRGSVQEQSRHGS
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RHSGIPRGQASSAVRDSRHWGSSGSQASDSEGHSEESDTQSVSGHGQAGPHQQSHQESARDR
SGGRSGRSGSFLYQVSTHEQSESAHGRTRTSTGRRQGSHHEQARDSSRHSASQEGQDTIRGHPG
SSRRGRQGSHYEQSVDRSGHSGSHHSHTTSQGRSDASRGQSGSRSASRQTRNDEQSGDGSRH
SWSHHHEASTQADSSRHSQSGQGQSAGPRTSRNQGSSVSQDSDSQGHSEDSERWSGSASRN
HRGSAQEQSRDGSRHPTSHHEDRAGHGHSAESSRQSGTHHAENSSGGQAASSHEQARSSAGE
RHGSHHQQSADSSRHSGIGHGQASSAVRDSGHRGSSGSQASDSEGHSEDSDTQSVSAHGQAG
PHQQSHQESTRGRSAGRSGRSGSFLYQVSTHEQSESAHGRAGPSTGGRQGSRHEQARDSSRHS
ASQEGQDTIRGHPGSRRGGRQGSYHEQSVDRSGHSGSHHSHTTSQGRSDASHGQSGSRSASR
ETRNEEQSGDGSRHSGSRHHEASTQADSSRHSQSGQGESAGSRRSRRQGSSVSQDSDSEAYPE
DSERRSESASRNHHGSSREQSRDGSRHPGSSHRDTASHVQSSPVQSDSSTAKEHGHFSSLSQD
SAYHSGIQSRGSPHSSSSYHYQSEGTERQKGQSGLVWRHGSYGSADYDYGESGFRHSQHGSVS
YNSNPVVFKERSDICKASAFGKDHPRYYATYINKDPGLCGHSSDISKQLGFSQSQRYYYY¶

Over 400 Histidine residues
Over 500 Glycine residues
Over 970 Serine residues = over all ~ 46%

Bacteria Interact with Filaggrin

Bacterial peptidases can degrade Filaggrin to small peptides and amino acids

Filaggrin has relatively short half life

Citrulline Histidine Serine Glycine
Major peptides, amino acids, gamma glu amino acids, deoxy fructosylated amino acids

- Citrullination of Filaggrin results in disassembly of Keratin fibers releasing free amino acids and regulating movement of Keratinocytes to outer barrier.
- Histidine – Component of Filaggrin, degrades to urocanic acid, pH regulation, anti-oxidant
- Serine – Key amino acid in Natural Moisturizing Factors (NMF)
- Glycine – Large part of collagen (1/3) – flexibility and firmness

Anti-oxidants in the dermis are Cysteine based

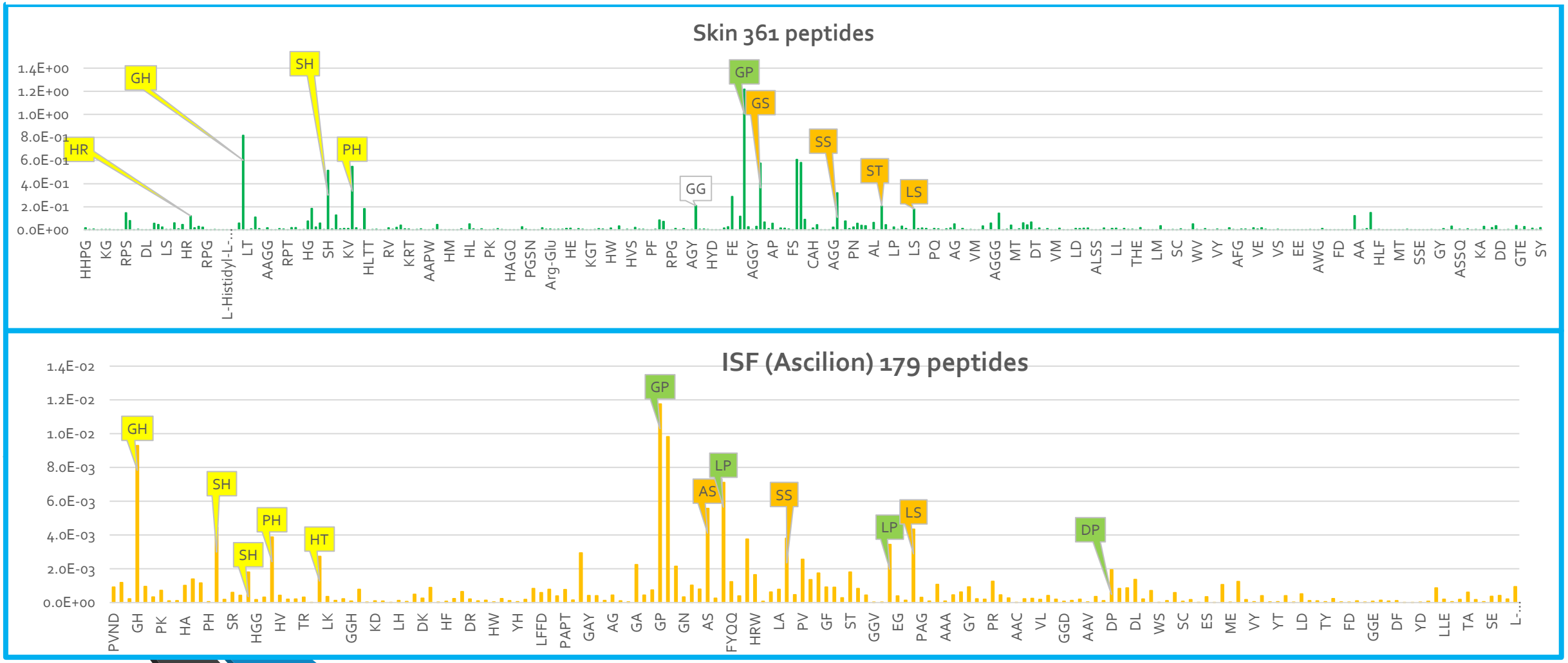
			Skin (HMT)	ISF (Ascilion)	plasma (Virtual Human)	Distal small intestine (Nimble)
dermis	Cystine	REDOX state	1.8E-02	1.2E-01	9.82E-02	7.91E-01
dermis	Taurine	Cys can convert to Taurine	1.8E-02	1.1E-01	2.83E-02	4.03E-02
dermis	Cys		7.6E-03	9.3E-04	1.44E-04	0.0E+00
dermis	Thiaproline	Nitrite scanvenger	5.4E-03	8.7E-04	6.36E-04	1.83E-02
dermis	γ-Glu-Taurine		2.5E-03	1.6E-04	6.34E-05	0.0E+00
dermis	Glutathione (GSH)		2.4E-03	4.4E-05	1.46E-07	0.0E+00
dermis	Hypotaurine	converts to Taurine	7.5E-04	2.0E-03	9.90E-04	0.0E+00
dermis	Glutathione (GSSG)		4.2E-04	3.2E-02	2.06E-04	1.78E-05
dermis	Cysteine glutathione disulfide	Converts to GSH	1.0E-04	2.9E-02	1.36E-03	
dermis	Homocysteic acid	excitatory amino acid	5.5E-05	0	0	0
dermis	L-Cysteinylglycine disulfide	Converts to GSH	4.7E-05	2.6E-02	0	2.83E-03
dermis	S-Acetyldihydrolipoamide	REDOX state	1.3E-05	0	3.5E-06	0

The major anti oxidants in the dermis are similar to those found in ISF, plasma and small intestine and contain the usual suspects (GSH, Cysteine,,,)

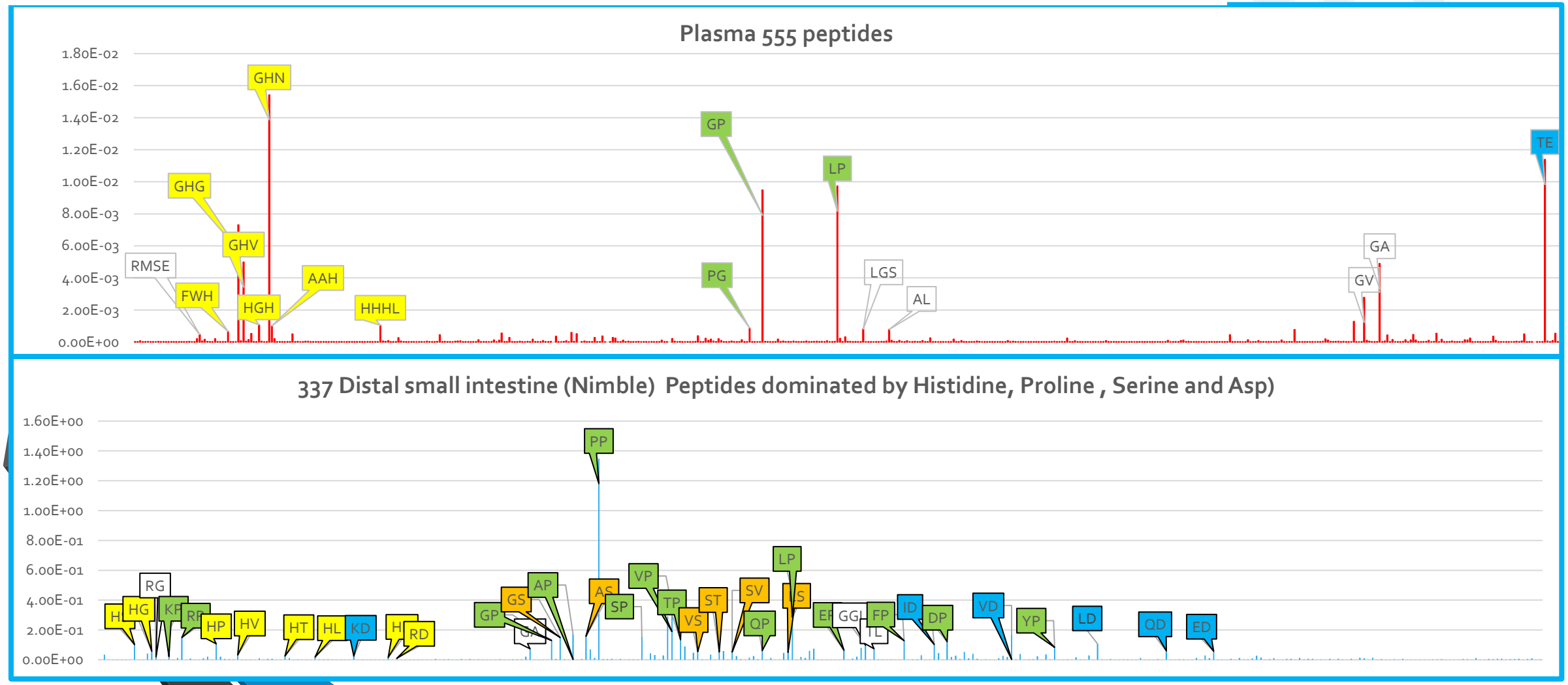
In summary, the anti-oxidant response in the epidermis is largely based on bioactive peptides that may in part be bacterial generated by proteolysis.

Feature	Histidine Peptides	Sulfur-Containing Peptides
Key Mechanism	Metal chelation: The imidazole ring of histidine binds to pro-oxidant metal ions like iron and copper, preventing them from generating free radicals via the Fenton reaction.	Direct radical scavenging: The thiol (-SH) group of cysteine and glutathione readily donates a hydrogen atom to neutralize free radicals, such as hydroxyl radicals and superoxide anions.
	Reactive species sequestration: Scavenges specific reactive species, including reactive nitrogen species (RNS) and reactive carbonyl species (RCS).	Redox regulation: Glutathione is a crucial component of the cell's main redox buffer, maintaining the reduced state of other antioxidants and proteins.
Stability	Generally more stable in biological systems because their antioxidant activity does not rely on a highly reactive thiol group.	The highly reactive nature of the thiol group makes these peptides susceptible to oxidation themselves. The glutathione system requires continuous regeneration to maintain its antioxidant function.
Targeting and Location	Carnosine and anserine have specific transporters, primarily from the proton-coupled oligopeptide transporter (POT) family, also known as the solute carrier 15 (SLC15) family. These dipeptides are transported both intact and as their constituent amino acids, beta -alanine and histidine, each of which also has its own transporters.	Glutathione is found ubiquitously within cells (intracellular) and is especially concentrated in the liver.
Regeneration	Some histidine peptides can be regenerated through interaction with other antioxidants, such as glutathione. Others may be generated by selective proteolysis.	Glutathione's oxidized form (GSSG) is enzymatically reduced back to its active form (GSH) by the enzyme glutathione reductase, a process that is essential for continuous function.
Role in Oxidative Stress	May function more as a long-term protective agent, activating other antioxidant pathways rather than immediately neutralizing all radicals.	A rapid-response, high-capacity antioxidant system that is essential for neutralizing oxidative threats under normal and stressed conditions.

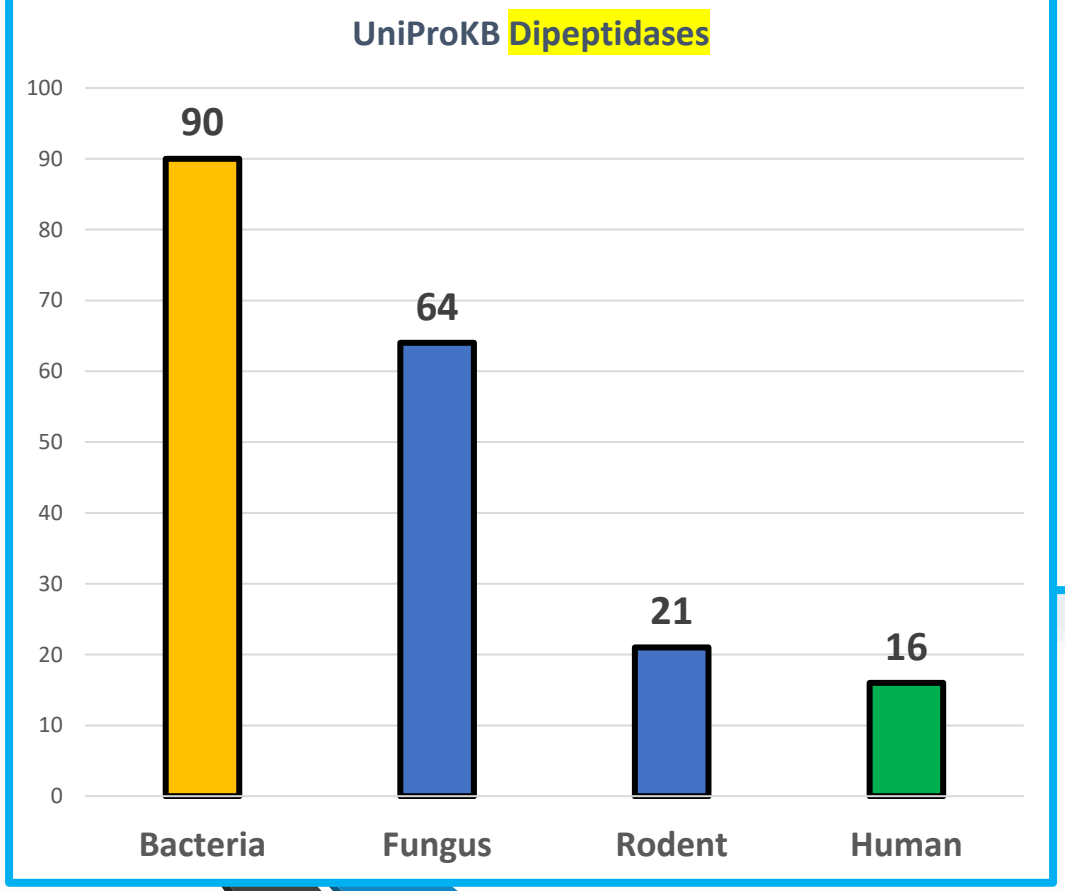
Peptide signatures are unique to each compartment, but share His Pro Ser peptides



Plasma tri and tetra His peptides greater than dipeptides
Small intestine –His Pro Ser Asp peptides



5 to 6 x more Dipeptidases in Bacteria than human Are these peptides from microbiome?



- Bacteria use proteases to eliminate misfolded or damaged proteins.
- Bacteria catabolize their own proteins, providing a nutrient source for the surviving bacterial population.
- Bacteria recycle components of their own cell wall, and reuse the amino acids to build new cell walls or use them for energy

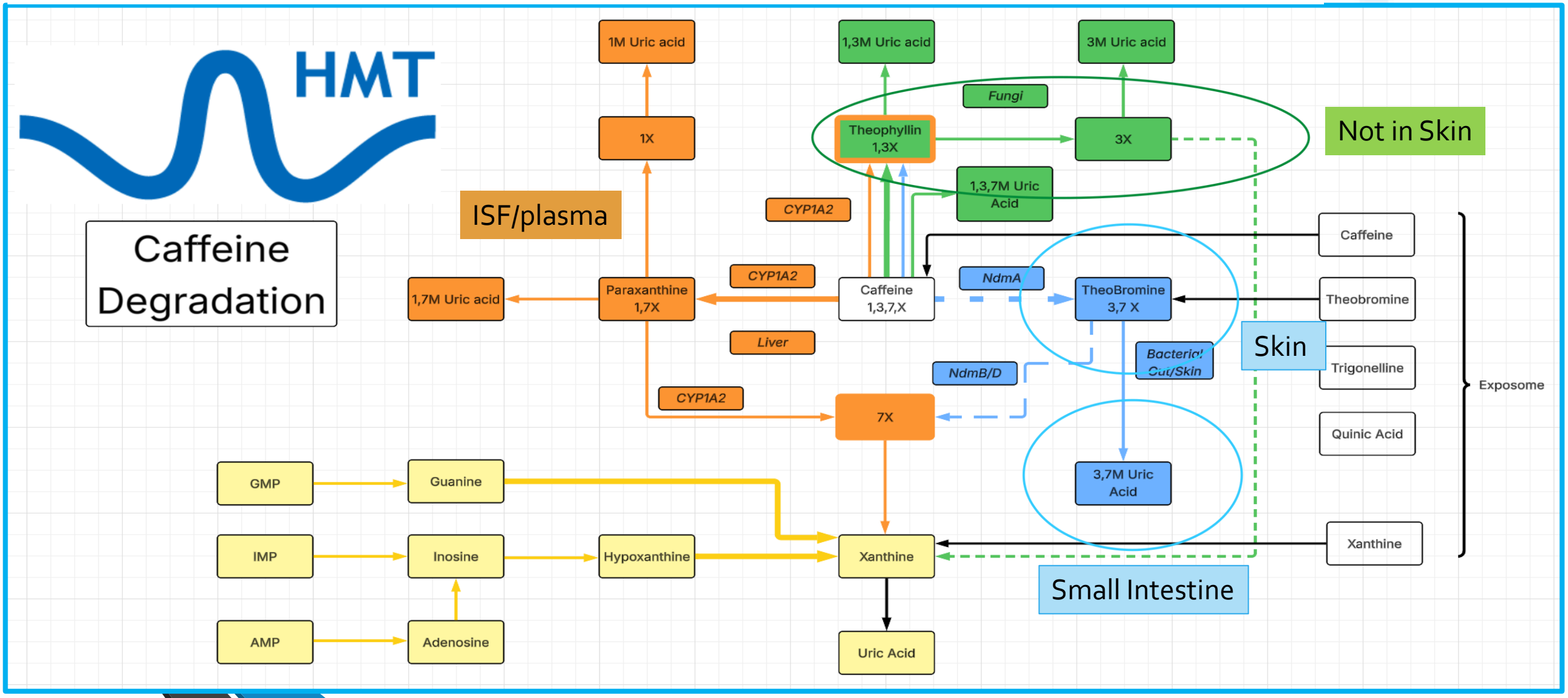
Source of small peptides – bacterial
Provide amino acids and bioactive

- Signaling molecules for cellular repair, antioxidant defenses, or anti-inflammatory responses
- Source of amino acids for human host
- Source of carbon and nitrogen for bacterial survival
- Epithelial turnover in skin and small intestine

Caffeine metabolism – link to microbiome

		Skin	ISF (Ascilion)	plasma (Virtual Human)	Distal small intestine (Nimble)
1-Methylxanthine	Human	0.0000	0	7.02E-05	0.00E+00
3,7-Dimethyluric acid	Bacterial	0	0	7.39E-06	1.6E-03
3-Methylxanthine	Fungal	0.00	2.6E-04	3.69E-04	6.30E-04
Paraxanthine	Human	4.0E-03	1.5E-02	8.5E-03	3.00E-03
Trigonelline	Exposome	1.4E-03	2.3E-02	2.7E-03	0.00E+00
Quinic Acid	Exposome	2.8E-03	9.9E-03	1.7E-03	4.34E-03
Theobromine	Bacterial / Exposome	1.7E-02	9.4E-03	9.1E-03	5.25E-03
Guanine	Purine catabolism	7.9E-03	1.9E-03	6.5E-06	8.69E-04
Caffeine	Exposome	3.8E-02	3.4E-02	1.0E-02	4.61E-03
Xanthine	Exposome	4.5E-03	5.0E-03	3.8E-04	1.88E-03
Hypoxanthine	Purine catabolism	6.4E-03	2.4E-02	5.0E-03	8.68E-04
1-Methyluric acid	Human	0.00	3.6E-04	3.96E-04	2.94E-05
Theophylline	Human	0	1.3E-03	1.3E-03	4.21E-04
7-Methylxanthine	Human	1.4E-04	8.3E-04	7.12E-04	1.57E-04
Uric acid	Pool	3.4E-02	2.3E-01	1.6E-01	6.96E-02

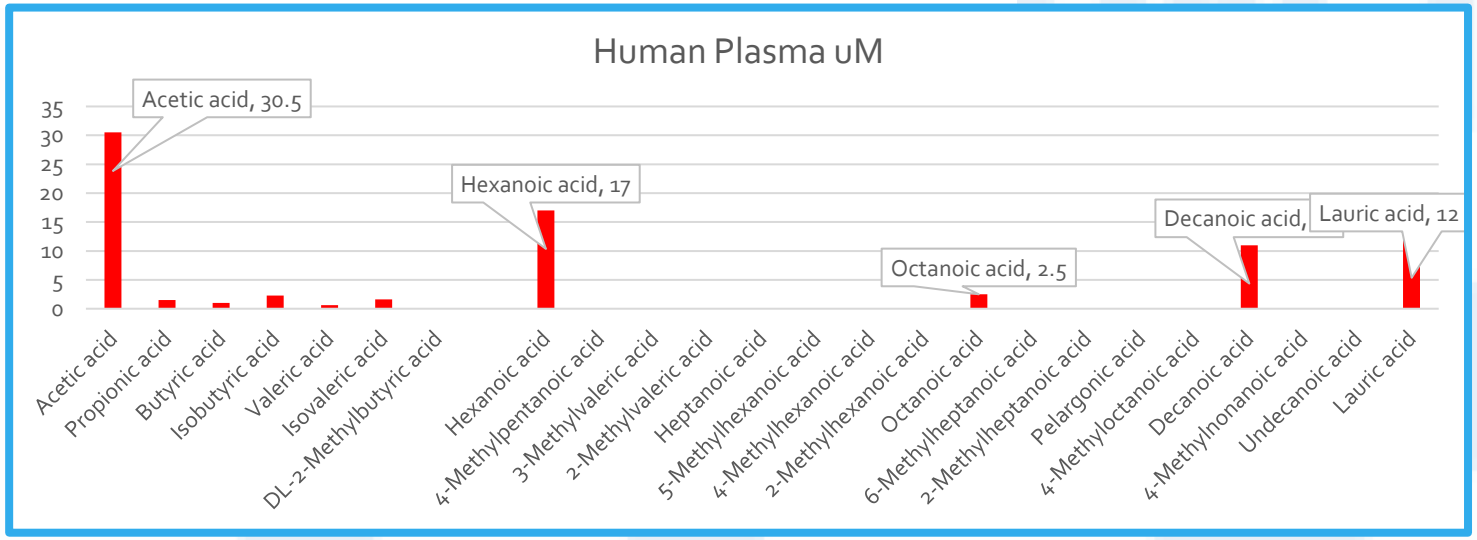
Exposome metabolism can be altered by changes in microbiome

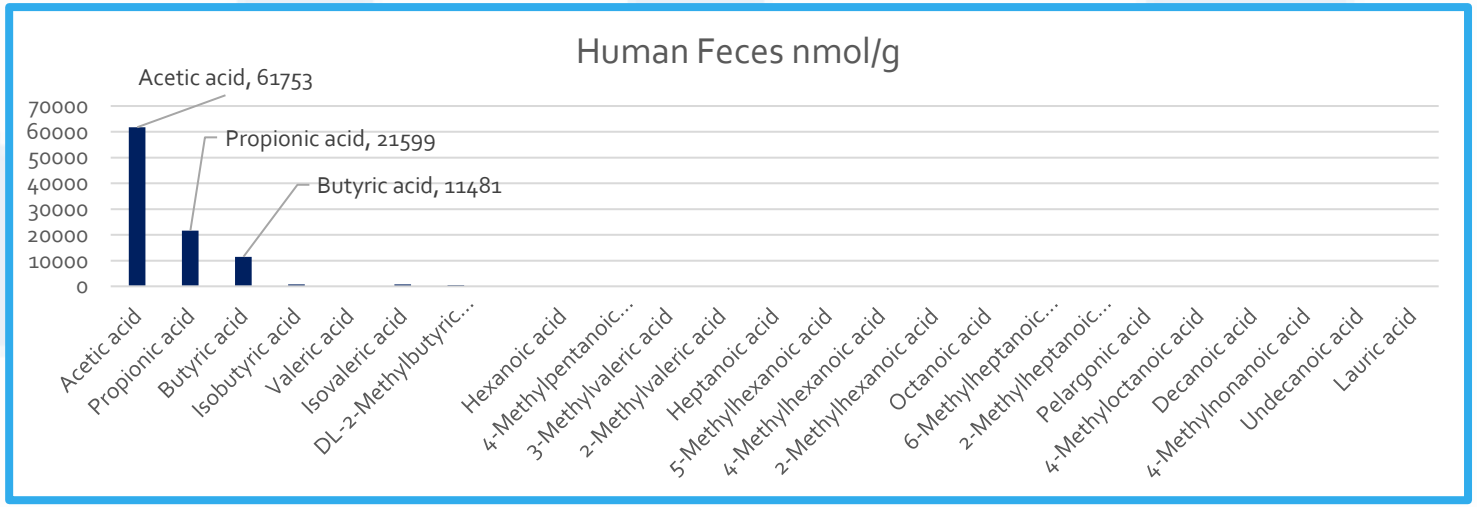


SCFA were not measured across all 4 matrices

HMT SC/MCFA Panel:

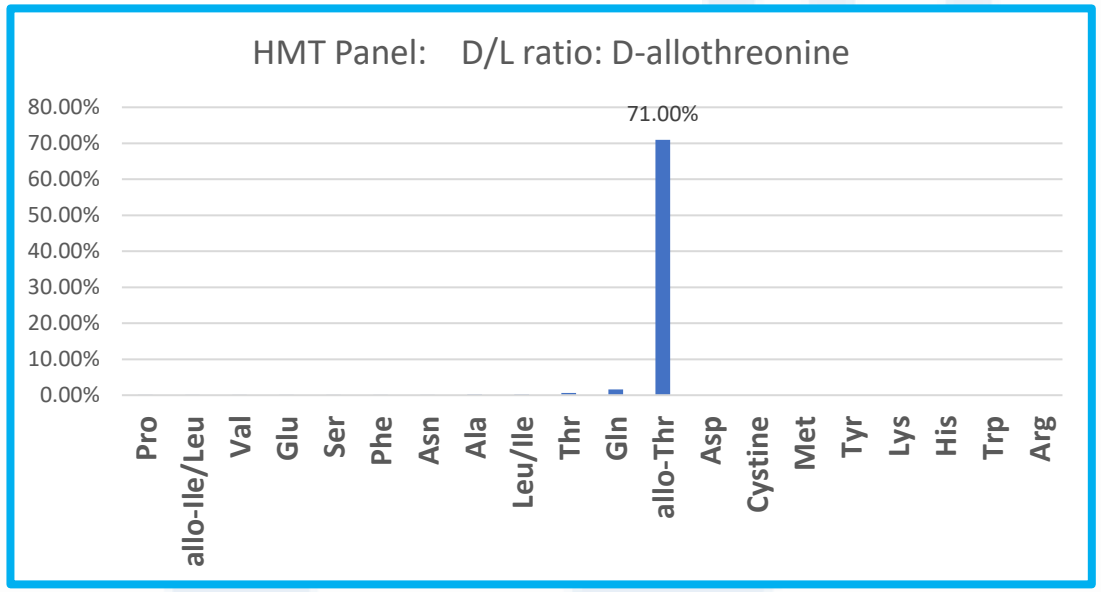
- 7 SCFAs
- 17 MCFAs
- Quantitative
- Multiple matrices





D vs L amino acids should be included

- 12 free D-AAs are produced by intestinal microbiota and identified bacterial groups.
- HMT D/L 20 amino acid panel
- Free D-AAs in the gut lumen may be involved in the cross-talk between gut bacteria and the host via binding to epithelial cell receptors and immune cells in the lamina propria.



BitBiome

bitBiome

- Single Cell sequencing of bacterial strains and mixtures
- Compared to WGS and 16sRNA sequencing, BitBiome provides SNP analysis allowing for strain variants & deeper dive into the complexity of the microbiome composition.
- "Exploring strain diversity of dominant human skin bacterial species using single-cell sequencing", K Ide et. al., Frontiers in Microbiology, August 5, 2022.
  - Provided 10-fold non-redundant strain genomes compared to shot gun metagenomic sequencing.
  - Explored skin commensal bacteria at the strain level.

What have we observed?

- 4 Different modalities to study microbial metabolites
- Each provide unique window into microbial signatures
- More than just SCFAs, Indoles to consider – modified amino acids, small peptides, many others
- Much we still do not understand about interplay microbiota and host

Hypotheses

1. The microbiome in distal small intestine determine amino acid composition in plasma and interstitial fluid. Microbiome controls amino acid composition using gamma glutamyl transferases.

2. Skin bacteria control amino acid concentrations and bioactive peptides by proteolysis of Filaggrin, Keratin and Collagen.

3. Deoxyfructosyl amino acids formed by microbiome in skin, liver and small intestine provide reservoir of C & N for bacteria and another pool for amino acids.

Go from here?

- Need systems biology approach to personalized medicine using orthogonal technologies
- Link different compartments – see how they communicate
- Study healthy people

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METABOLOMICS SOCIETY

https://metabolomicssociety.org