Nutritional Status Assessment SMO

SM Smith
MA Heer
SR Zwart

Schedule

- 3 pre-flight blood/urine collections (L-180, L-45, L-10)
- 5 in-flight blood/urine collections
  - FD15, FD30, FD60, FD120, FD180
- 2 post-flight blood/urine collections (R+0, R+30)

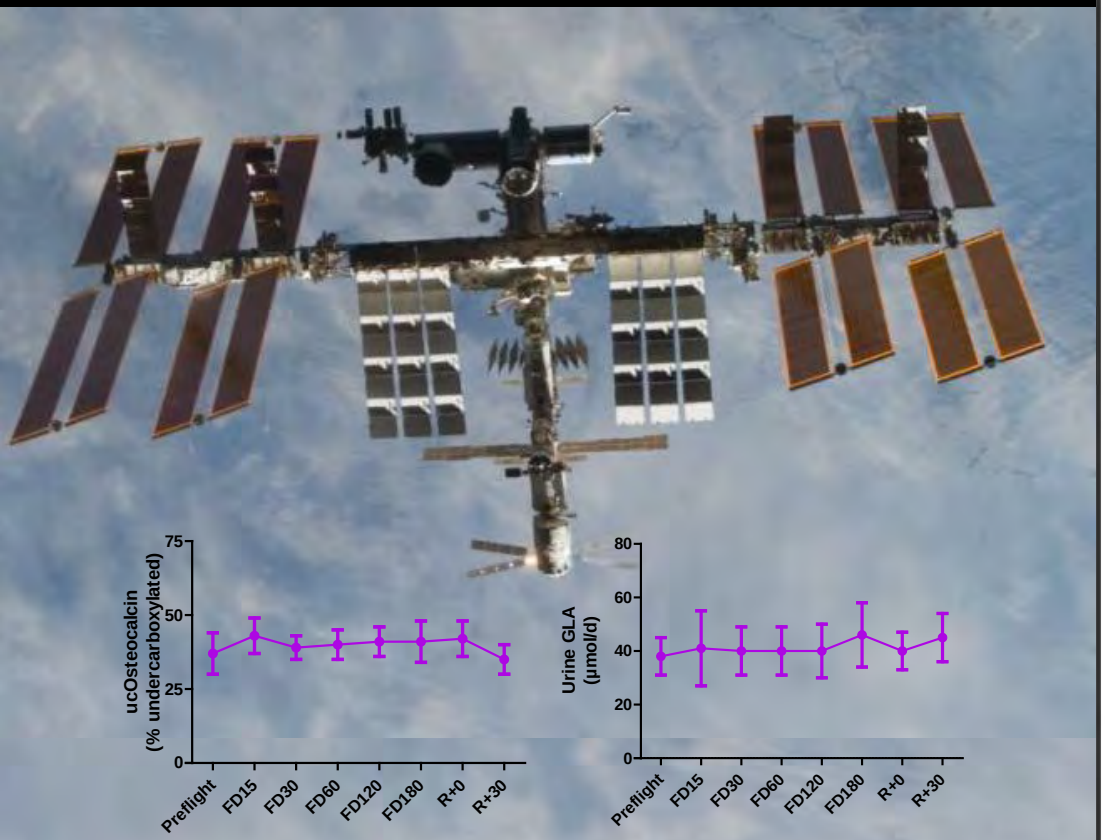
Samples are analyzed for a battery of tests

- Vitamins
- Bone Markers
- Antioxidants/Ox Damage

Minerals
Hormones
General Chemistry

Proteins
Renal Stone Risk
Cytokines

Vitamin K



ORIGINAL ARTICLE

JBMR

Vitamin K Status in Spaceflight and Ground-Based Models of Spaceflight

Sara R Zwart,[1] Sarah L Booth,[2] James W Peterson,[2] Zuwei Wang,[3] and Scott M Smith[4]

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[2]Jean Mayer USDA Human Nutrition Center on Aging at Tufts University, Boston, MA, USA
[3]Enterprise Advisory Services, Inc., Houston, TX, USA
[4]Human Adaptation and Countermeasures Division, NASA Lyndon B. Johnson Space Center, Houston, TX, USA

ABSTRACT

Bone loss is a well-documented change during and after long-duration spaceflight. Many types of countermeasures to bone loss have been proposed, including vitamin K supplementation. The objective of this series of studies was to measure change in vitamin K status in response to microgravity under a variety of spaceflight and spaceflight analog (model) conditions, including long-duration spaceflight studies ($n = 15$), three bed rest studies ($n = 15$, 49, and 24), and a 14-day saturation dive ($n = 6$). In crew members who flew 2–6 months on the International Space Station, in-flight and postflight plasma phylloquinone concentrations were unchanged from the preflight mean. Consistent with this finding, urinary γ-carboxyglutamic acid (GLA), a measure of vitamin K-dependent protein turnover, did not change in response to flight. Serum undercarboxylated osteocalcin (%ucOC), a measure of vitamin K function, was generally unchanged in response to flight. Spaceflight findings were corroborated by findings of no changes in phylloquinone, urinary GLA, or %ucOC during or after bed rest in three separate bed rest studies (21–90 days in duration) or after a 14-day saturation dive. The data presented here do not support either a need for vitamin K supplementation during spaceflight or the suggestion of using vitamin K as a bone loss countermeasure in spaceflight. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: VITAMIN K; BONE LOSS; BONE TURNOVER MARKERS; SPACEFLIGHT; BED REST

Introduction

Bone loss is a seemingly inevitable outcome of spaceflight.[1–4] Of the many types of bone loss countermeasures evaluated to date, none have been proven effective during flight.[5] Vitamin K is a nutrient linked to bone health and as such has been suggested as a potential countermeasure. Regardless of its efficacy in preventing bone loss, if the vitamin K status of astronauts were established as suboptimal during spaceflight, vitamin K supplementation might be warranted.

Vitamin K is an enzyme cofactor for the production of γ-carboxyglutamic acid (GLA) residues in specific proteins (GLA proteins). GLA proteins are involved in a number of regulatory functions, including bone mineralization. When vitamin K status is suboptimal, the assumption is that GLA proteins are not fully carboxylated and will thus be less effective. Osteocalcin is a GLA protein that is synthesized by osteoblasts and is thought to have a role in regulation of bone mineralization. Undercarboxylated osteocalcin (ucOC) provides a good measure of vitamin K functional status, with specific regard to bone.[6]

Vitamin K has been included as a concern for the nutritional status of astronauts because of uncertainty about the efficiency of vitamin K synthesis by the gastrointestinal flora in microgravity and about potentially altered absorption in this unique environment.[5] A known and consistent occurrence in the spaceflight environment is loss of bone mineral. Data showing a role for vitamin K status and/or vitamin K supplementation in bone health on Earth has led some to suggest that vitamin K treatment could play a role in protecting bone against microgravity-induced mineral loss.[7,8]

Three studies provide evidence that vitamin K status of astronauts may be suboptimal during spaceflight and suggest that vitamin K supplementation may be warranted. Carboxylation of osteocalcin was decreased in two cosmonauts during both short- and long-duration spaceflight, and it returned to preflight levels soon after landing.[9] In a case study with in-flight vitamin K supplementation, a "pharmacological dose" of 10 mg vitamin K1/day decreased undercarboxylated osteocalcin to preflight levels.[9,10] Concomitant with the positive changes in vitamin K status with supplementation, urinary calcium tended

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DOI: 10.1002/jbmr.289
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Vitamin K status does not appear affected by spaceflight (or bed rest).

Testosterone

STS-55/D-2 (1993)

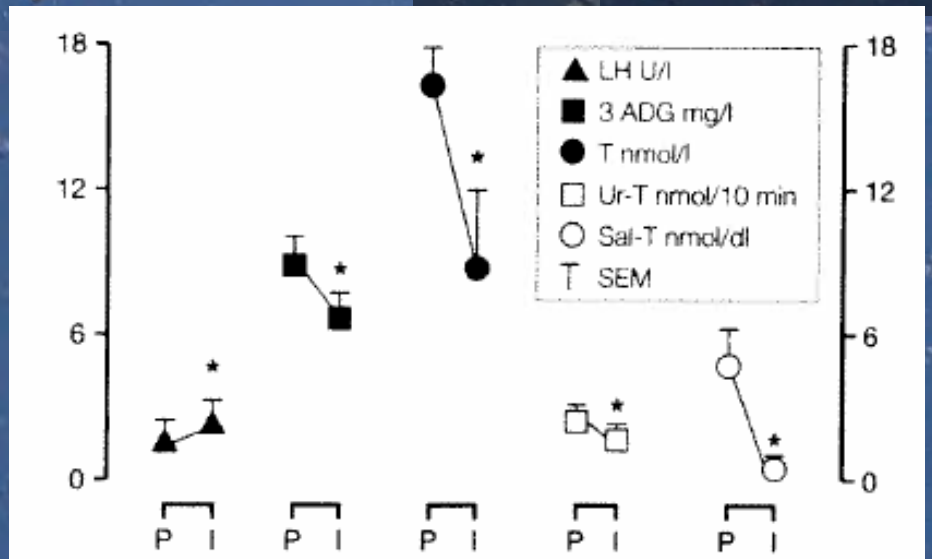
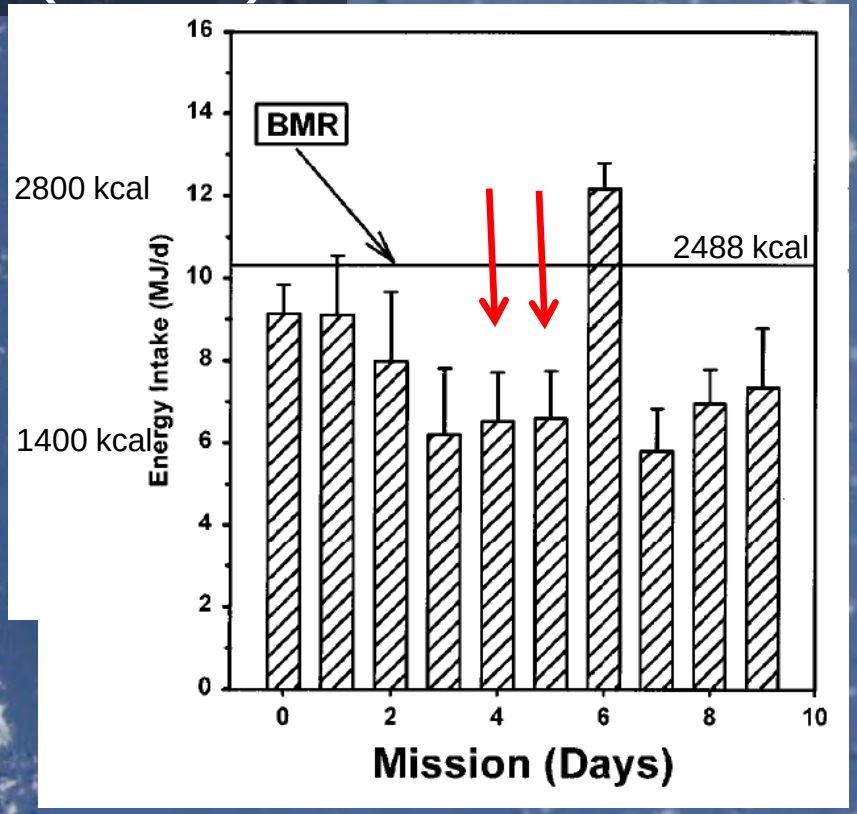


Fig. 1 - Changes in the hypothalamic-pituitary-gonadal axis (HPGA)-related hormone concentrations in 4 male astronauts participating into the D2 mission in 1993. Means and SEM's are reported. *refers to statistical significance (at the 95% level) of differences between pre-flight (P) and in-flight levels (I); Sal-T= salivary T; Ur-T= urinary T; ADG: androstendial glucuronide.



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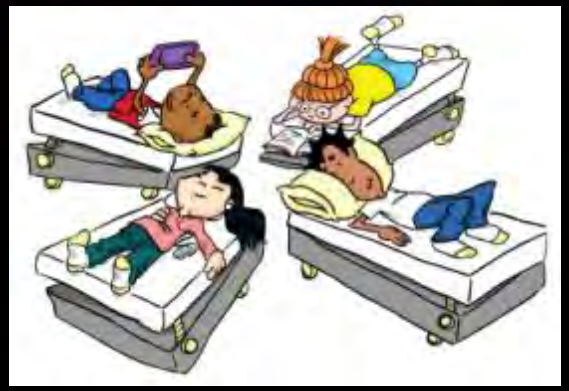

J Clin Endocrin Metab. First published ahead of print November 2, 2011 as doi:10.1210/jc.2011-2233

ORIGINAL ARTICLE

Endocrine Research

Long-Duration Space Flight and Bed Rest Effects on Testosterone and Other Steroids

Scott M. Smith, Martina Heer, Zuwei Wang, Carolyn L. Huntoon, and Sara R. Zwart

Space Life Sciences Directorate (S.M.S., C.L.H.), Johnson Space Center, National Aeronautics and Space Administration, Enterprise Advisory Services, Inc. (Z.W.), and Universities Space Research Association (S.R.Z.), Houston, Texas 77058; Department of Nutrition and Food Science (M.H.), Nutritional Physiology, University of Bonn, D-53117 Bonn, Germany; Profil Institute for Metabolic Research GmbH (M.H.), D-41460 Neuss, Germany

Context: Limited data suggest that testosterone is decreased during space flight, which could contribute to bone and muscle loss.

Objective: The main objective was to assess testosterone and hormone status in long- and short-duration space flight and bed rest environments and to determine relationships with other physiological systems, including bone and muscle.

Design: Blood and urine samples were collected before, during, and after long-duration space flight. Samples were also collected before and after 12- to 14-d missions and from participants in 30- to 90-d bed rest studies.

Setting: Space flight studies were conducted on the International Space Station and before and after Space Shuttle missions. Bed rest studies were conducted in a clinical research center setting. Data from Skylab missions are also presented.

Participants: All of the participants were male, and they included 15 long-duration and nine short-duration mission crew members and 30 bed rest subjects.

Main Outcome Measures: Serum total, free, and bioavailable testosterone were measured along with serum and urinary cortisol, serum dehydroepiandrosterone, dehydroepiandrosterone sulfate, and SHBG.

Results: Total, free, and bioavailable testosterone was not changed during long-duration space flight but were decreased ($P < 0.01$) on landing day after these flights and after short-duration space flight. There were no changes in other hormones measured. Testosterone concentrations dropped before and soon after bed rest, but bed rest itself had no effect on testosterone.

Conclusions: There was no evidence for decrements in testosterone during long-duration space flight or bed rest. (***J Clin Endocrinol Metab* 97: 0000–0000, 2012**)

The recent National Research Council review of the life sciences research programs at the National Aeronautics and Space Administration noted that "although exposure to microgravity *per se* triggers bone and muscle loss because of the reduction in mechanical loading forces, losses are likely exacerbated by additional factors in the space environment (*e.g.* altered nutrition, hormonal disruptions, psychological stress). Thus, the development of effective countermeasure strategies will require input from experts across multiple disciplines (*e.g.* basic bone and muscle biologists, cardiovascular physiologists, endocrinologists, exercise physiologists, nutritionists, biomecha-

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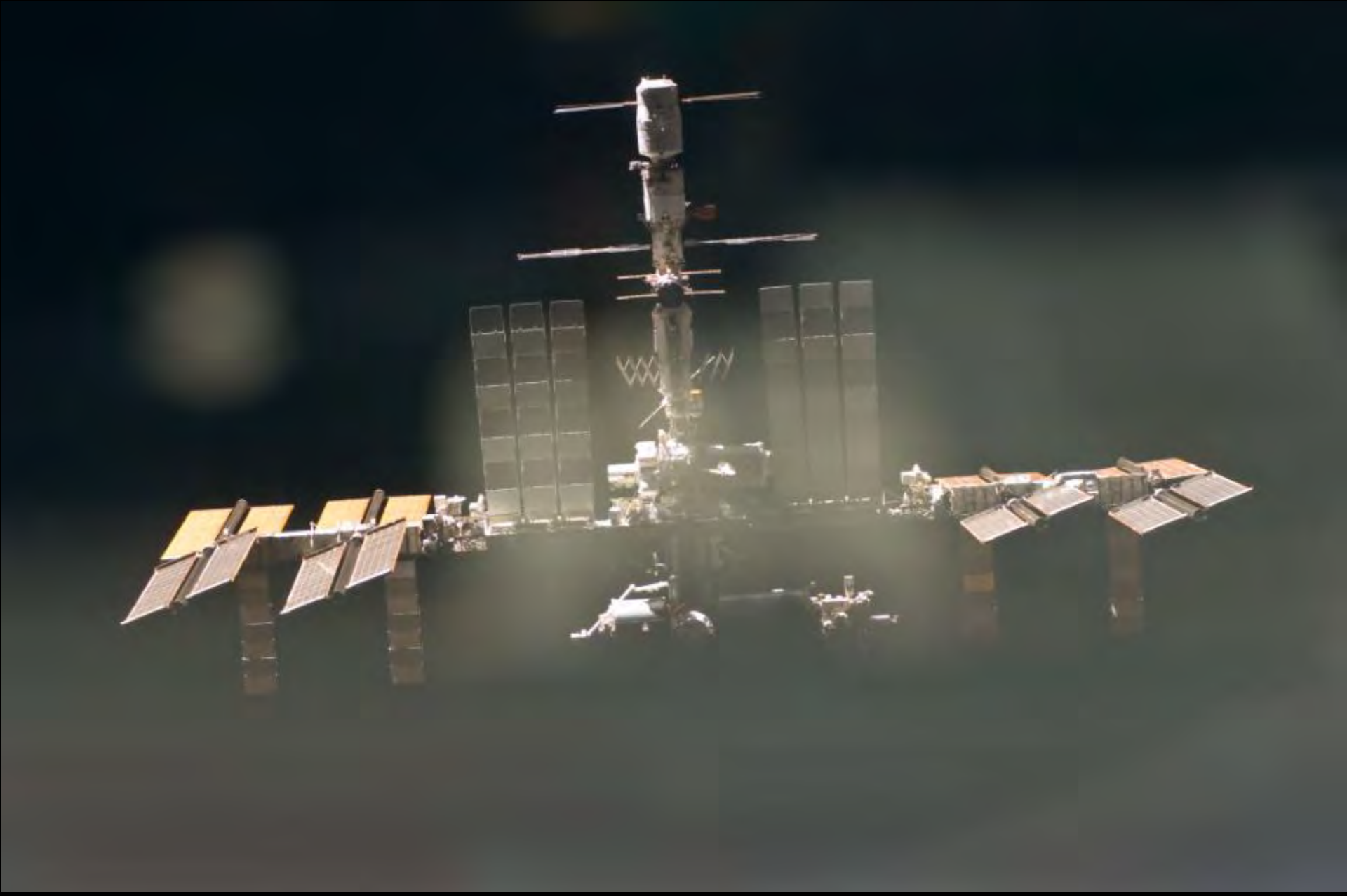
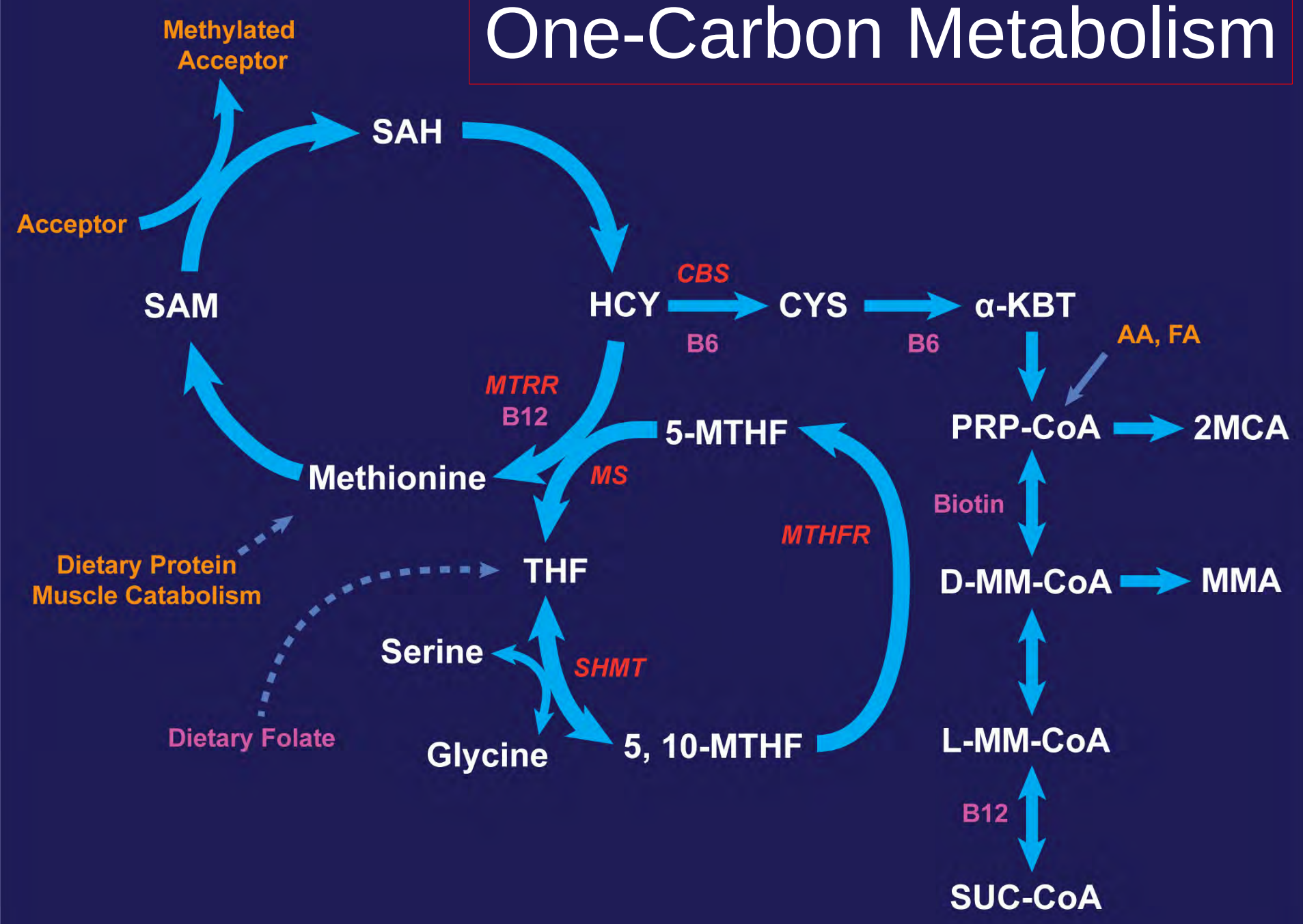
doi: 10.1210/jc.2011-2233 Received August 4, 2011. Accepted October 6, 2011.

Abbreviations: aRED, Advanced resistive exercise device; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; FD, flight day; LC-MS/MS, liquid chromatography-tandem mass spectrometry; R, return.

Testosterone does not appear to be affected by spaceflight (or bed rest).



One-Carbon Metabolism

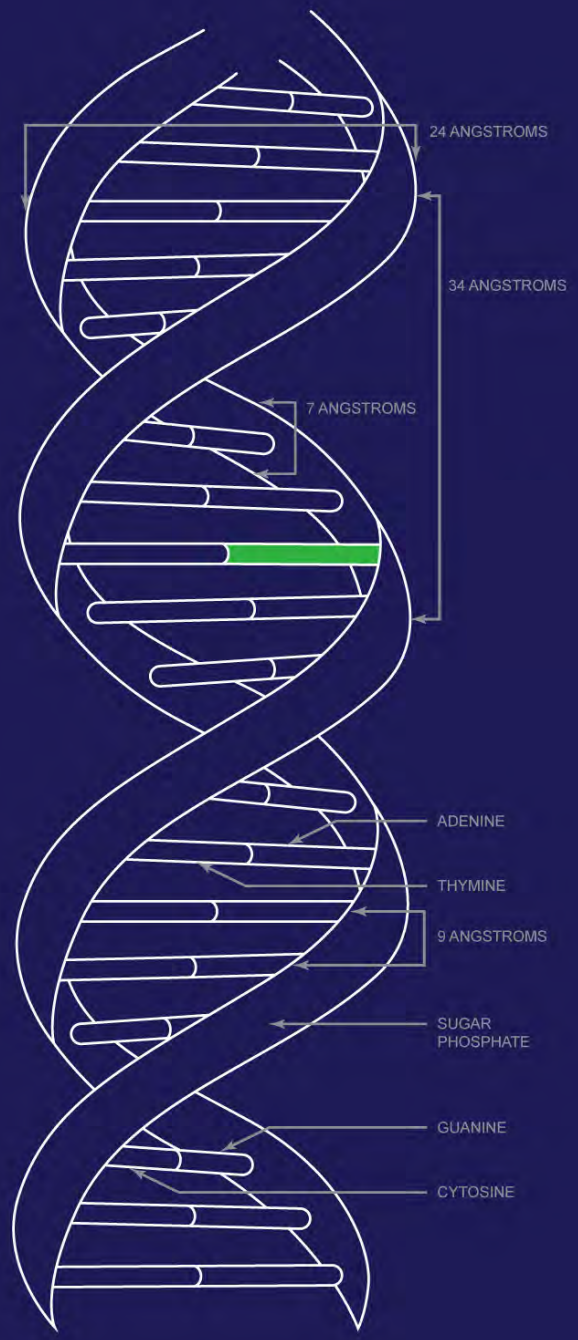


Enzymes

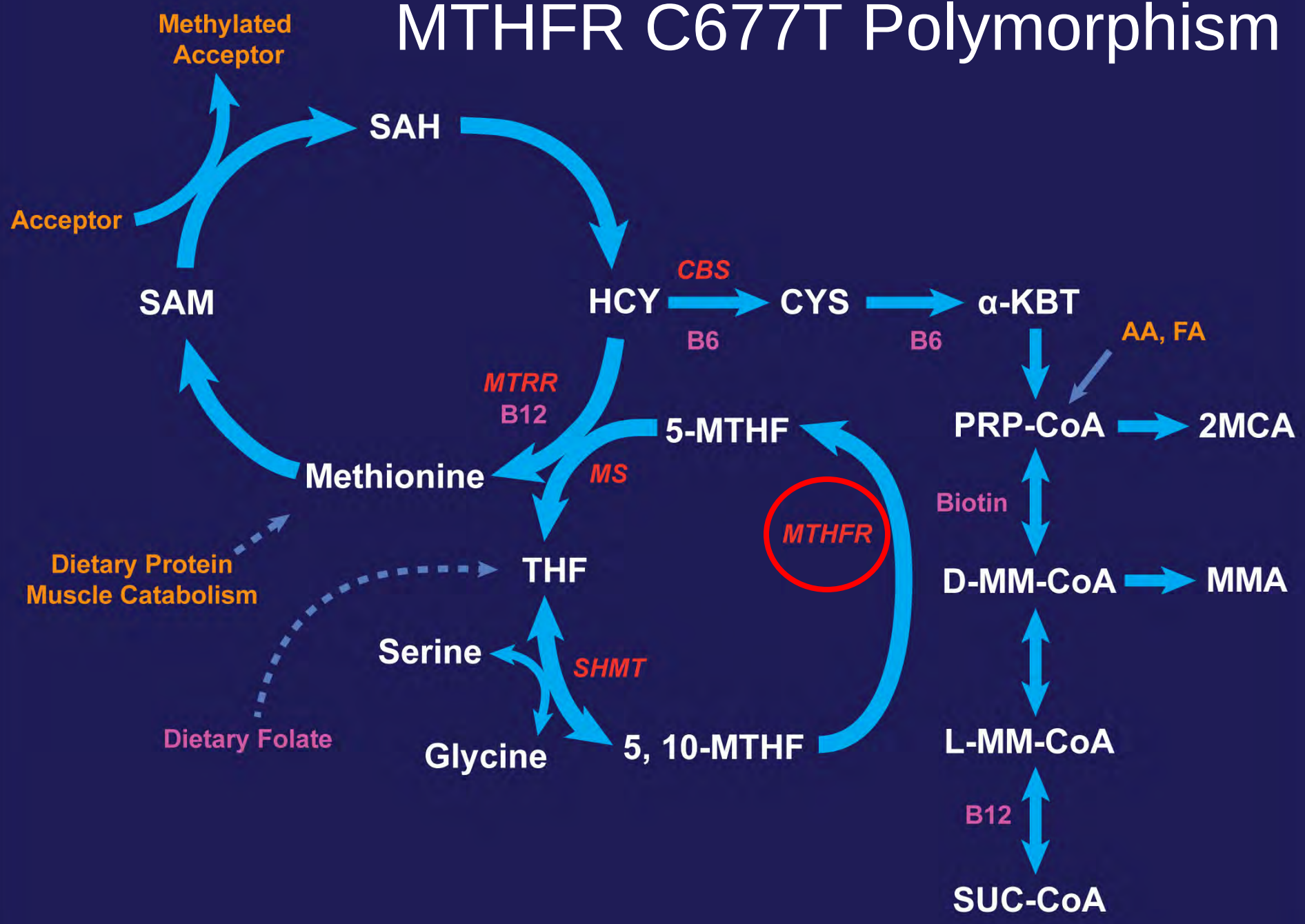
- Proteins, amino acid strings
- catalyze reactions
- assembled from amino acids based on “blueprints”

Enzyme Polymorphisms

- poly = multiple, “morph” = forms
- For many (all?) enzymes, there are small differences in blueprints across the population (e.g., blood types)

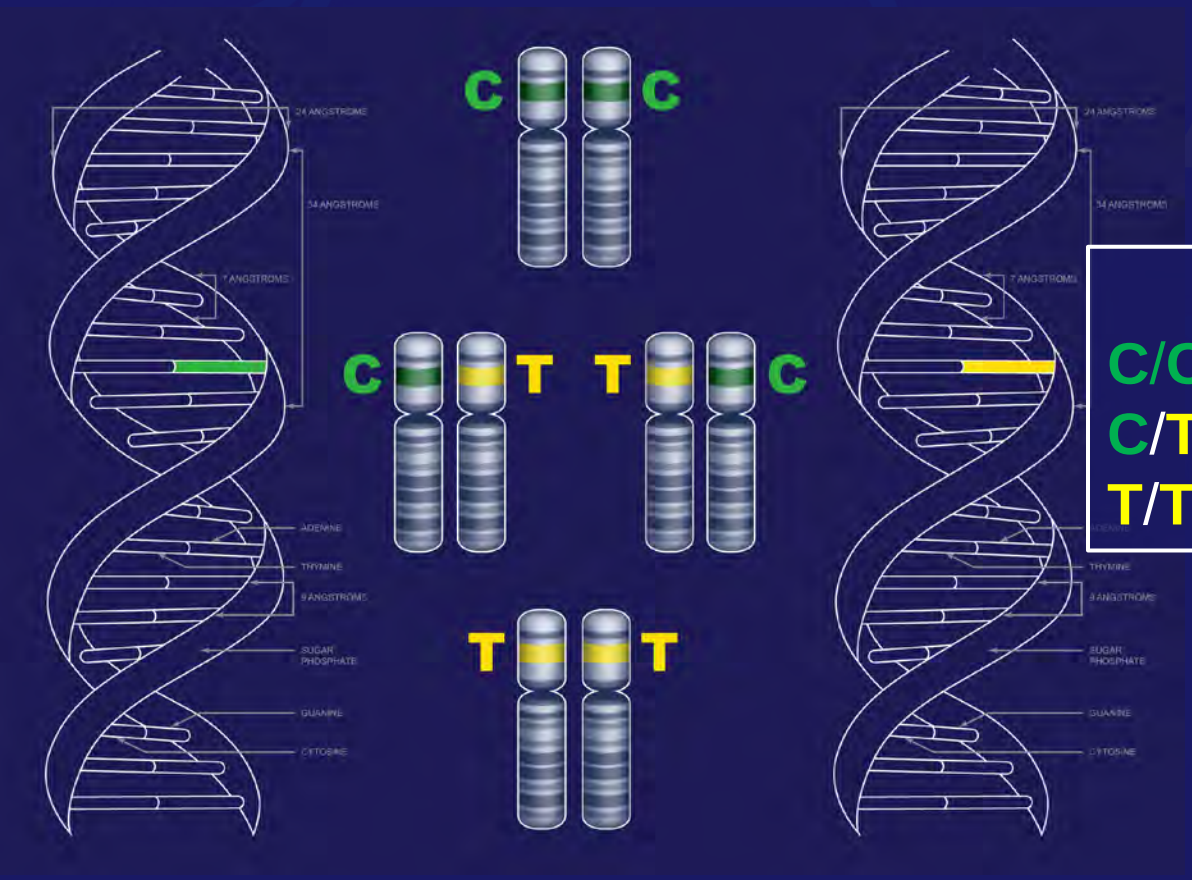


MTHFR C677T Polymorphism

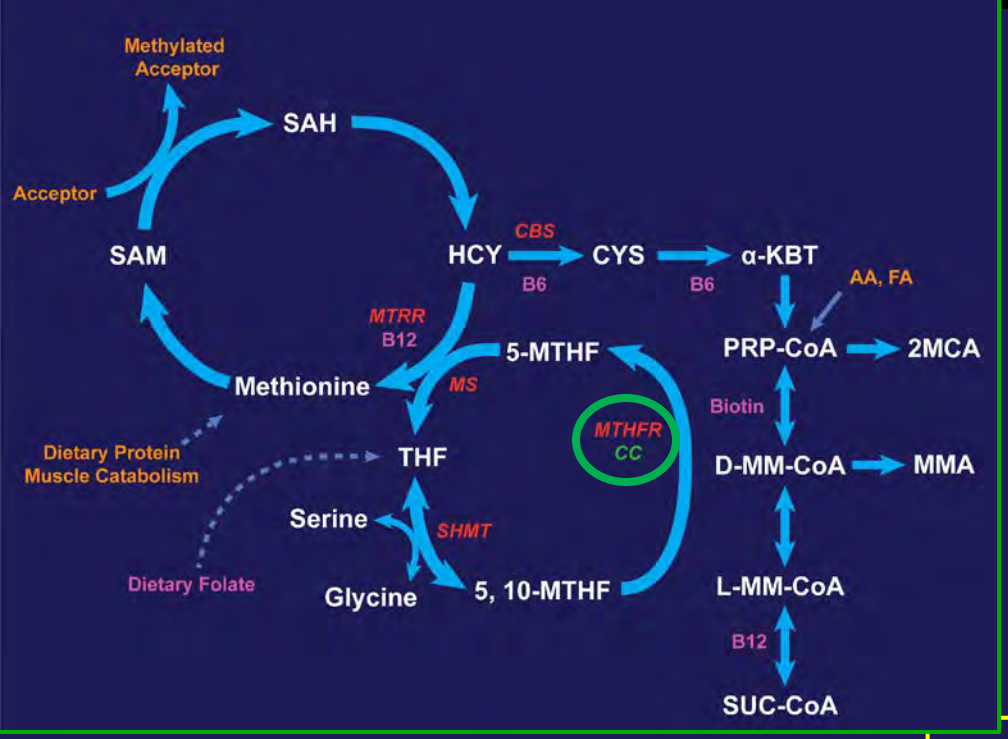


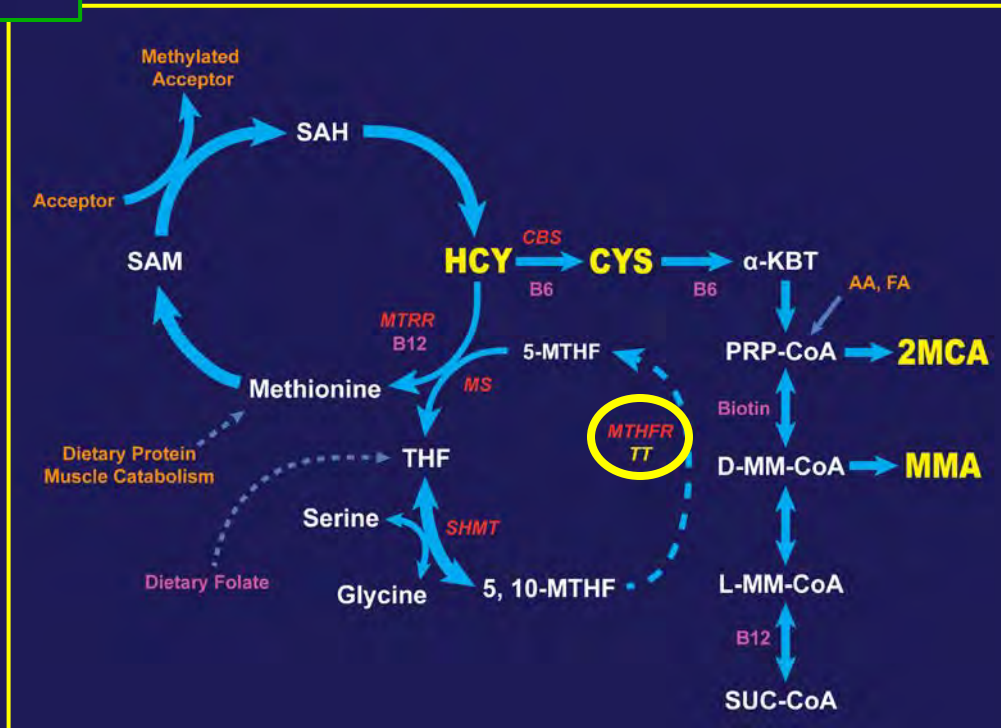
MTHFR C677T Polymorphism

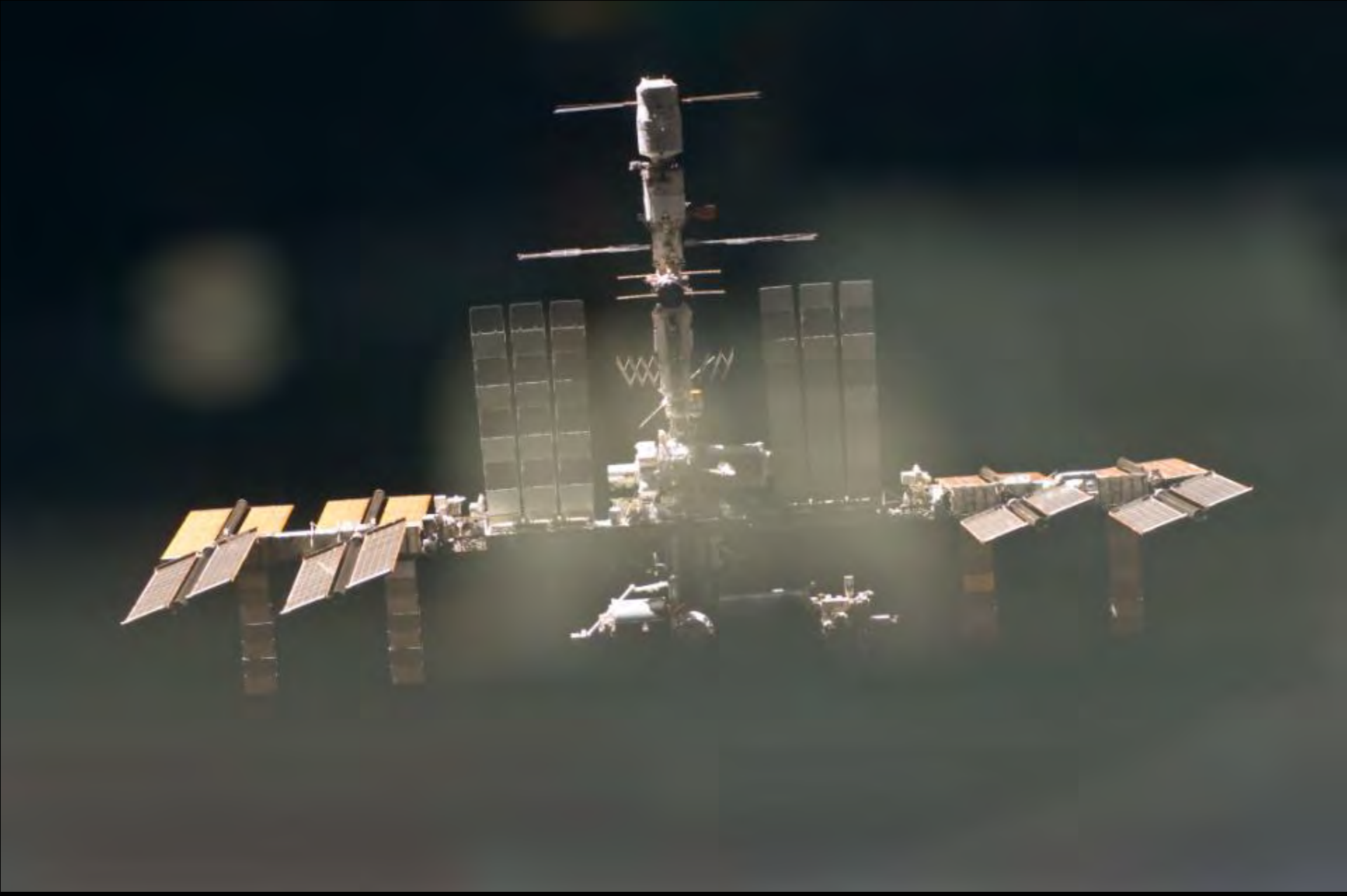
Everybody has 2 sets of blueprints (mom and dad), resulting in four possibilities of this polymorphism.

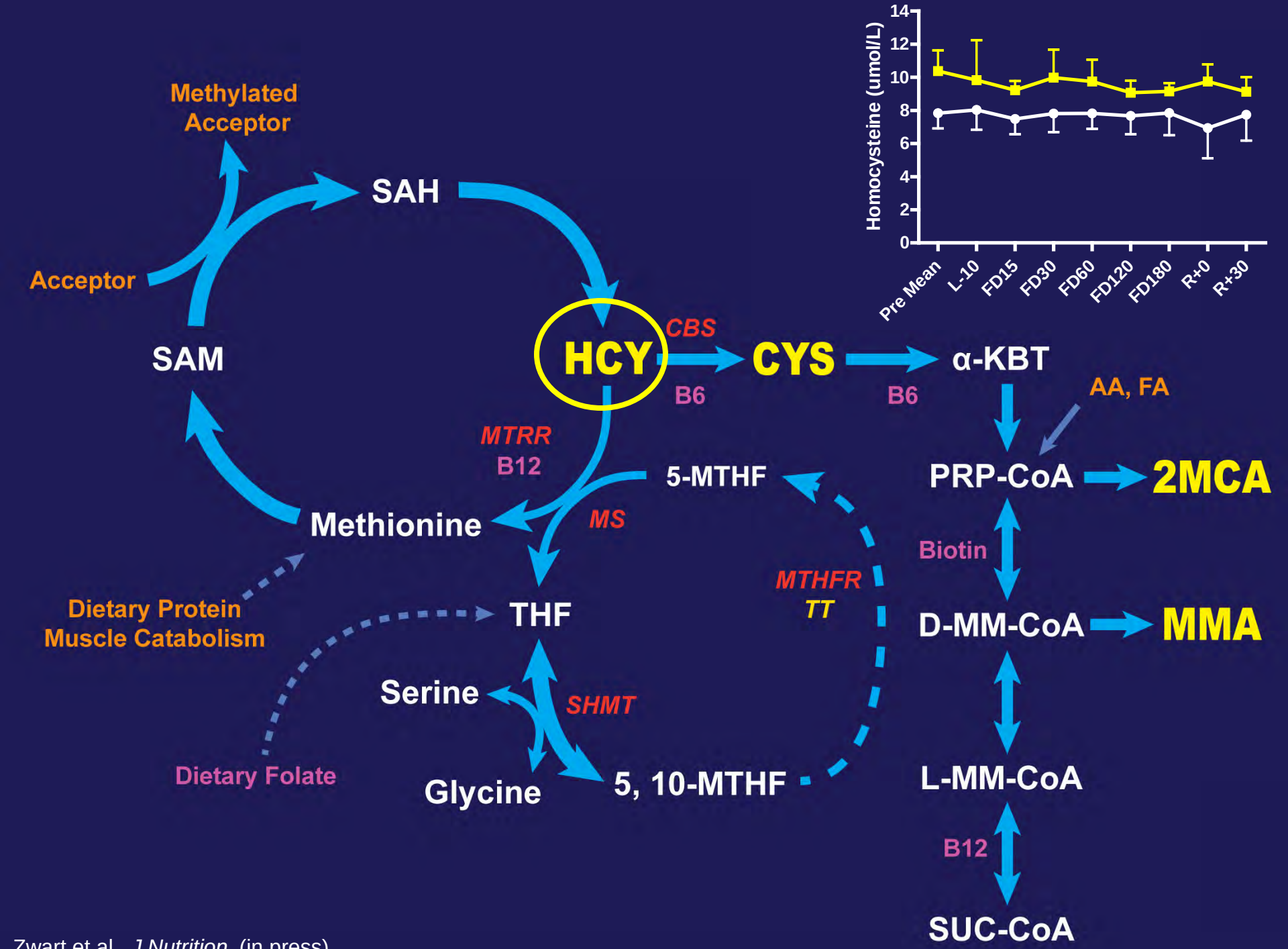


	% Pop	Enz Act.
C/C	~35%	100%
C/T(T/C)	~50%	~66%
T/T	~15%	~50%

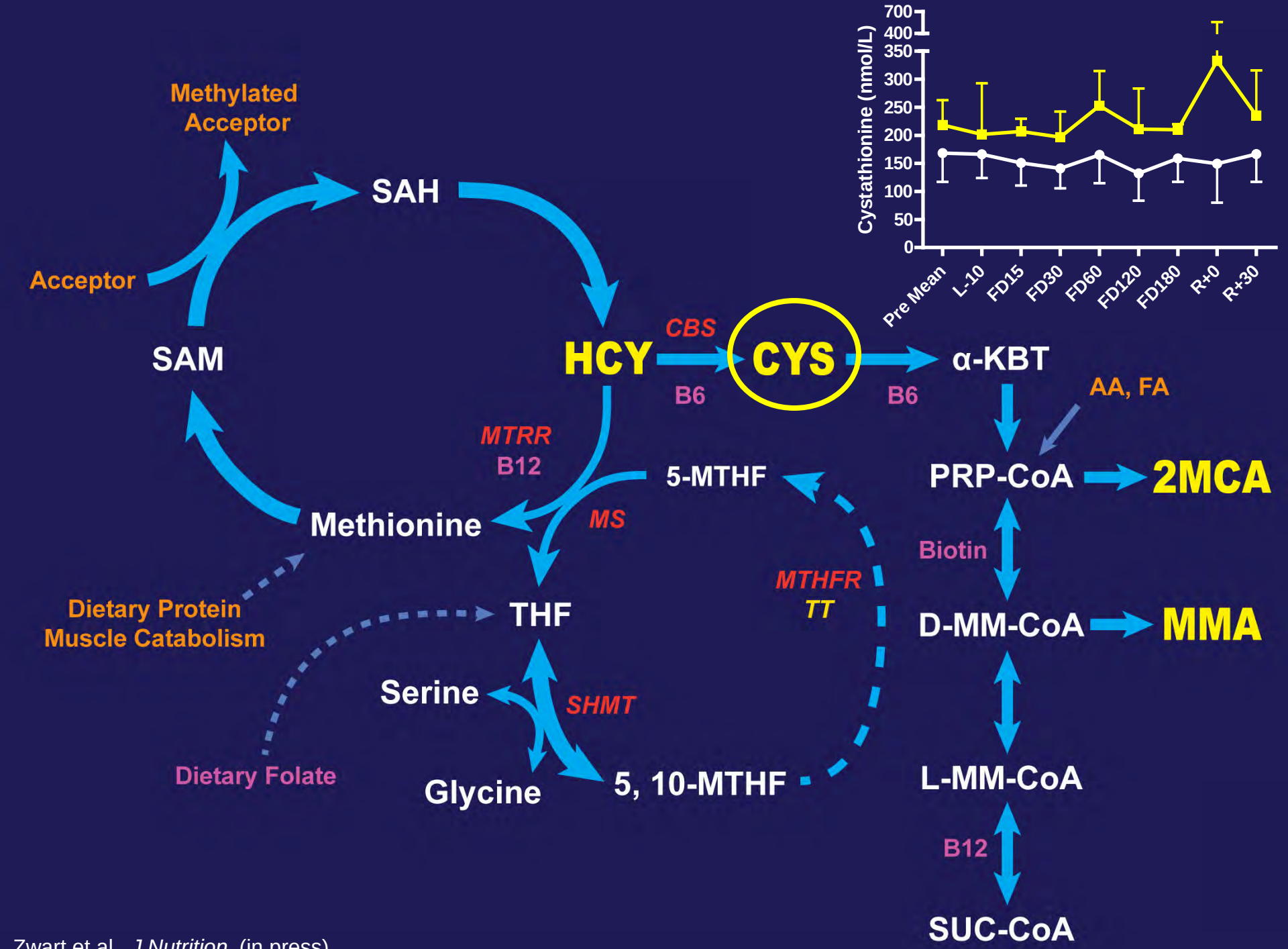

Methylated Acceptor
SAH
Acceptor
SAM
HCY
CBS
B6
CYS
B6
α-KBT
AA, FA
MTRR
B12
5-MTHF
PRP-CoA
2MCA
Methionine
MS
Biotin
MTHFR
CC
Dietary Protein
Muscle Catabolism
THF
D-MM-CoA
MMA
Serine
SHMT
Dietary Folate
Glycine
5, 10-MTHF
L-MM-CoA
B12
SUC-CoA


Methylated Acceptor
SAH
Acceptor
SAM
HCY
CBS
B6
CYS
B6
α-KBT
AA, FA
MTRR
B12
5-MTHF
PRP-CoA
2MCA
Methionine
MS
Biotin
MTHFR
TT
Dietary Protein
Muscle Catabolism
THF
D-MM-CoA
MMA
Serine
SHMT
Dietary Folate
Glycine
5, 10-MTHF
L-MM-CoA
B12
SUC-CoA

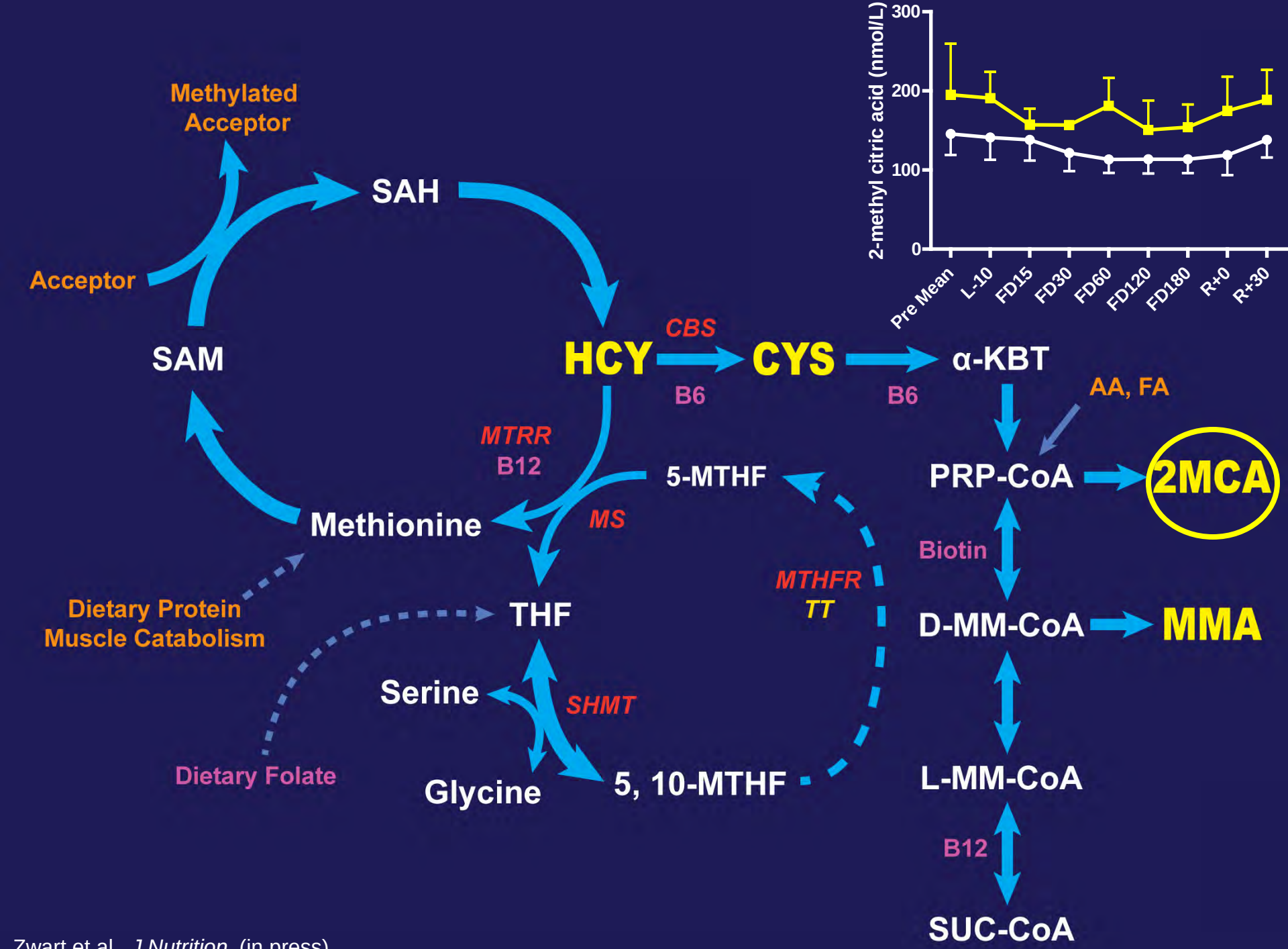




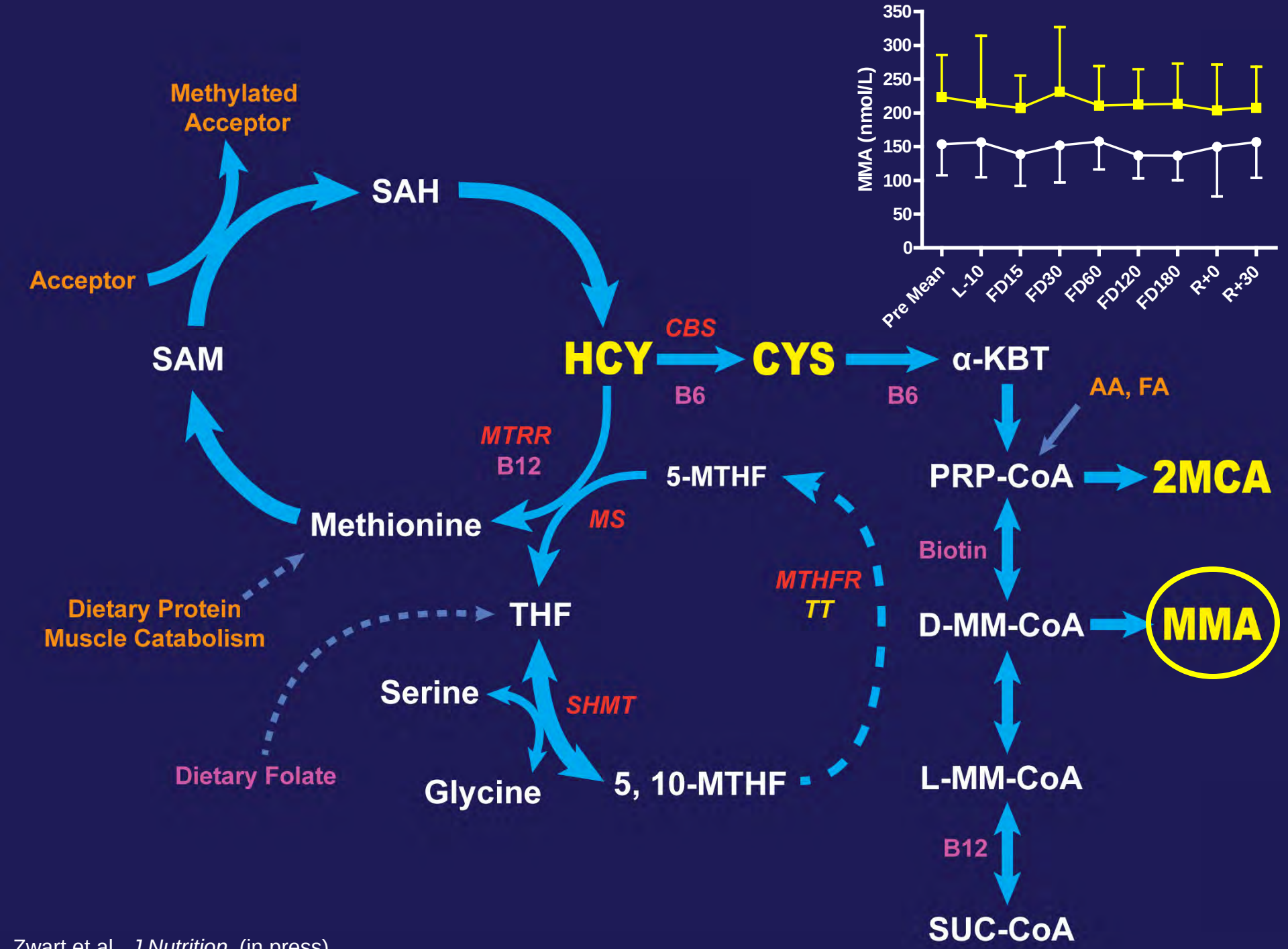
Zwart et al., *J Nutrition* (in press)



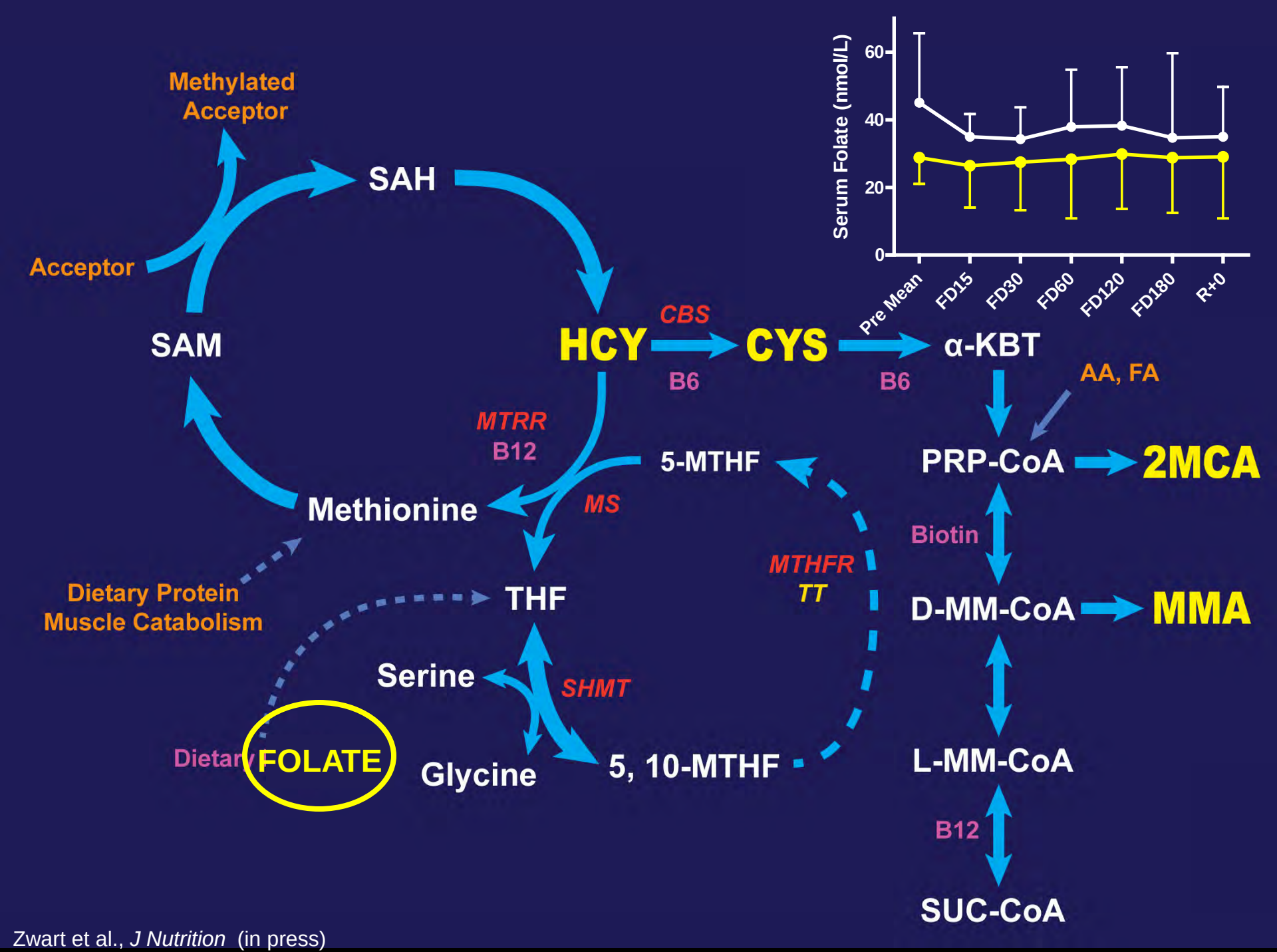
Zwart et al., *J Nutrition* (in press)



Zwart et al., *J Nutrition* (in press)

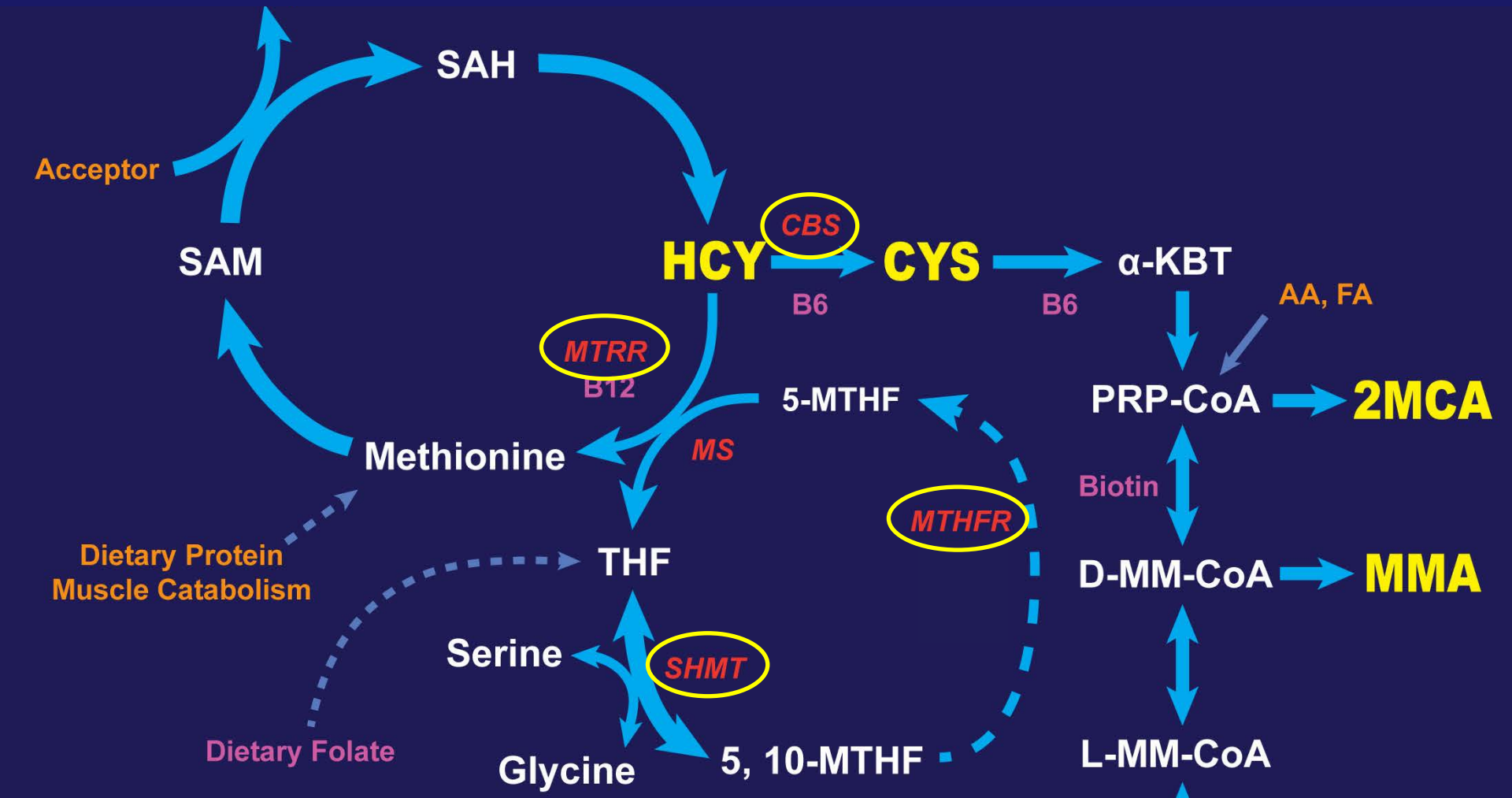


Zwart et al., *J Nutrition* (in press)

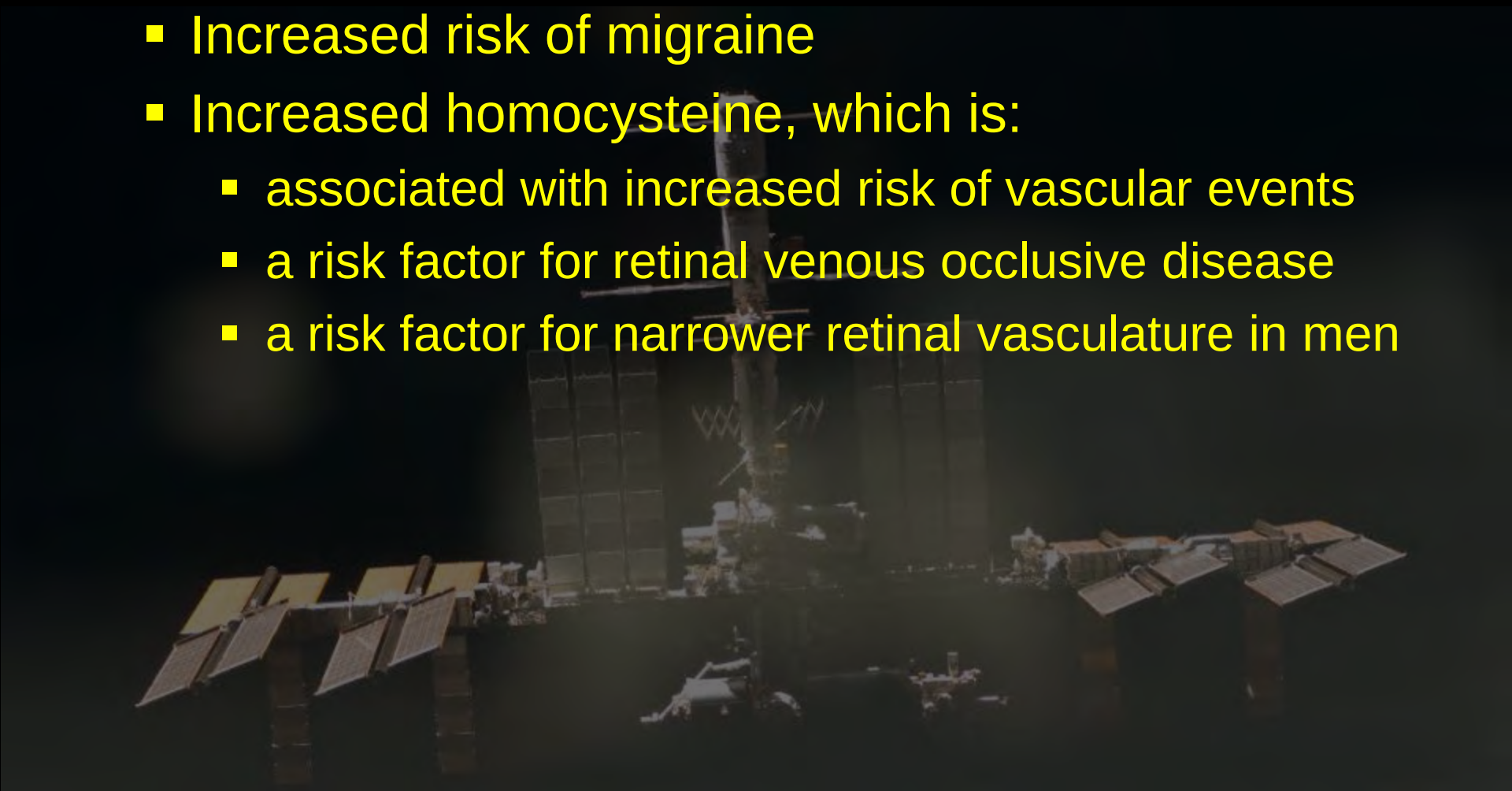


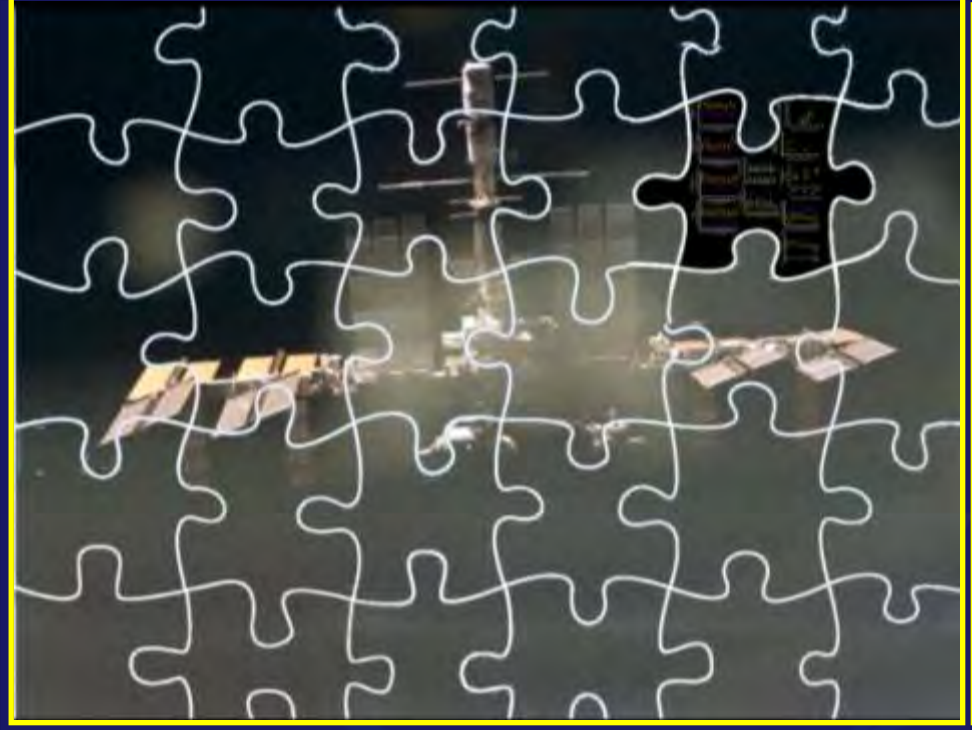
Zwart et al., *J Nutrition* (in press)

These data strongly suggest a role for polymorphism(s) of one or more of the enzymes in this pathway in spaceflight-related vision changes.



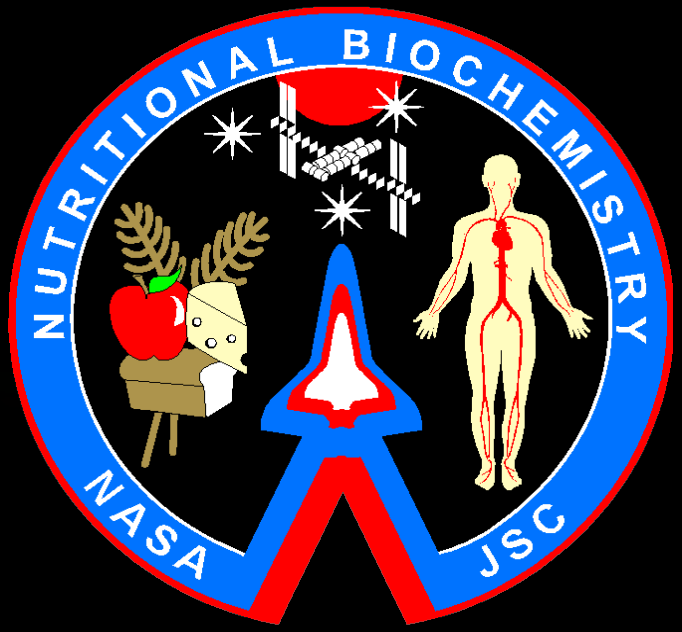
- MTHFR polymorphisms associated with:
  - Increased risk of stroke
  - Increased risk of migraine
  - Increased homocysteine, which is:
    - associated with increased risk of vascular events
    - a risk factor for retinal venous occlusive disease
    - a risk factor for narrower retinal vasculature in men





Forward Work

- Proposal to follow up submitted to HHC in March 2011…
- Results will:
  - Inform risks
  - Inform therapeutic options
  - Inform VIIP research
  - Inform countermeasure options/application



The Journal of Nutrition. First published ahead of print February 1, 2012 as doi: 10.3945/jn.111.154245.

The Journal of Nutrition
Biochemical, Molecular, and Genetic Mechanisms

Vision Changes after Spaceflight Are Related to Alterations in Folate– and Vitamin B-12–Dependent One-Carbon Metabolism[1,2]

Sara R. Zwart,[3] C. Robert Gibson,[4] Thomas H. Mader,[5] Karen Ericson,[6] Robert Ploutz-Snyder,[3] Martina Heer,[7] and Scott M. Smith[8]*

[3]Division of Space Life Sciences, Universities Space Research Association, Houston, TX; [4]Wyle Science, Technology and Engineering Group, Houston, TX, and Coastal Eye Associates, Webster, TX; [5]Alaska Native Medical Center, Anchorage, AK; [6]Department of Chemistry, Indiana University-Purdue University Fort Wayne, Fort Wayne, IN; [7]University of Bonn, Bonn, Germany, and Profil Institute for Metabolic Research GmbH, Neuss, Germany; and [8]Human Adaptation and Countermeasures Division, Space Life Sciences Directorate, National Aeronautics and Space Administration Johnson Space Center, Houston, TX

Abstract

Approximately 20% (7 of 38) of astronauts on International Space Station (ISS) missions have developed measurable ophthalmic changes after flight. This study was conducted to determine if the folate– and vitamin B-12–dependent 1-carbon metabolic pathway is altered in these individuals. Since 2006, we have conducted experiments on the ISS to evaluate nutritional status and related biochemical indices of astronauts before, during, and after flight. Data were modeled to evaluate differences between individuals with ophthalmic changes ($n = 5$) and those without them ($n = 15$), all of whom were on ISS missions of 48–215 d. We also determined whether mean preflight serum concentrations of the 1-carbon metabolites and changes in measured cycloplegic refraction after flight were associated. Serum homocysteine (Hcy), cystathionine, 2-methylcitric acid (2MCA), and methylmalonic acid concentrations were 25–45% higher ($P < 0.001$) in astronauts with ophthalmic changes than in those without them. These differences existed before, during, and after flight. Preflight serum concentrations of Hcy and cystathionine, and mean in-flight serum folate, were correlated with change (postflight relative to preflight) values in refraction ($P < 0.05$), and preflight serum concentrations of 2MCA tended to be associated ($P = 0.06$) with ophthalmic changes. The biochemical differences observed in crewmembers with vision issues strongly suggest that their folate– and vitamin B-12–dependent 1-carbon transfer metabolism was affected before and during flight. The consistent differences in markers of 1-carbon metabolism between those who did and those who did not develop changes in vision suggest that polymorphisms in enzymes of this pathway may interact with microgravity to cause these pathophysiologic changes. J. Nutr. doi: 10.3945/jn.111.154245.

Introduction

In what has been described as one of the most important clinical findings from spaceflight to date, several astronauts on long-duration ISS[9] missions have had long-term (and potentially permanent) changes in vision during and after spaceflight (1). To date, 38 astronauts from the Canadian Space Agency, European Space Agency, Japan Aerospace Exploration Agency, and NASA have lived aboard the ISS for 3–6 mo. Seven of them have had measurable ophthalmic changes after flight, including optic disc edema, globe flattening, choroidal folds, hyperopic shifts, and cotton wool spots. These 7 cases have been described in detail (1).

Currently, the etiology of these ophthalmic changes is unknown, but it has been hypothesized that microgravity-induced cephalad fluid shifts and resultant increased intracranial pressure and/or localized intraorbital changes may be involved (1). We present here evidence that the folate– and vitamin B-12–dependent 1-carbon metabolic pathway involving Hcy recycling and transsulfuration may be different in these individuals. This presents an alternative hypothesis that individuals with this altered pathway may be predisposed to anatomic and/or physiologic changes that render them susceptible to ophthalmologic damage during spaceflight.

Participants and Methods

All protocols were approved by the Johnson Space Center Committee for the Protection of Human Subjects, and informed consent was obtained from all participants. Participants ($n = 20$) were crewmembers on Expeditions 14–25 (missions of 48–215 d, 153 ± 52 d, mean ± SD;

[1] Supported by the NASA Human Research Program.
[2] Author disclosures: S. R. Zwart, C. R. Gibson, T. H. Mader, K. Ericson, R. Ploutz-Snyder, M. Heer, and S. M. Smith, no conflicts of interest.
[9] Abbreviations used: Hcy, homocysteine; ISS, International Space Station; 2MCA, 2-methylcitric acid; MMA, methylmalonic acid; MTHFR, 5,10-methylenetetrahydrofolate reductase; OC+, group of individuals who had ophthalmic changes; OC-, group of individuals who did not have ophthalmic changes; R+0, landing day; R+30, 30 d after landing.
* To whom correspondence should be addressed. E-mail: scott.m.smith@nasa.gov.

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doi: 10.3945/jn.111.154245.

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JN THE JOURNAL OF NUTRITION

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A Comparison of Markers of Nutritional Status and Oxidative Stress in Rats Exposed to High Dietary Iron and Radiation, with Markers in Mice Flown on A 14 day Shuttle Mission (STS-135)

Jennifer L.L. Morgan, Corey A. Theriot, Honglu Wu, Scott M. Smith, and Sara R. Zwart

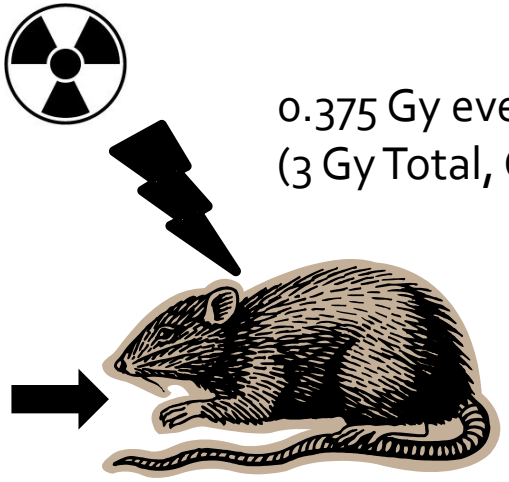
Human Research Program
Investigators Workshop
2012

Why Radiation and High Fe?

- Radiation exposure will only increase as space flights move beyond low earth orbit
- Astronauts experience an increase in total body Fe from two sources
  - Decrease in red blood cell mass
  - High dietary Fe from eating processed foods
- Low level radiation and increase total body Fe, independently, are known to cause can independently increase oxidative damage, resulting in protein, lipid and DNA oxidation
- Oxidative stress increases the risk of many health problems including cancer, cataracts, and heart disease.

Rats Exposed to High Fe Diet and Radiation

Animals Arrived	High Fe diet	Radiation exposure	Termination
-20	0	14	30
20 days	14 days	16 days	

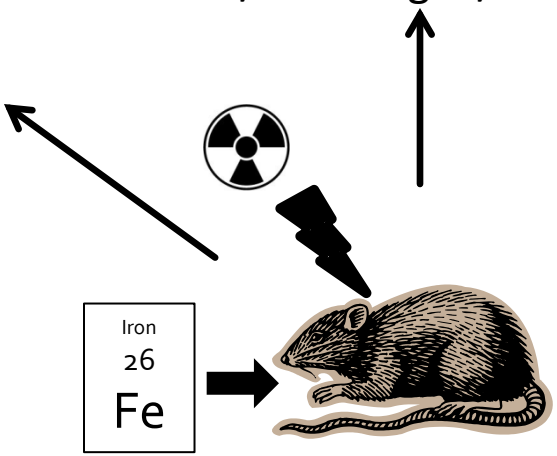


0.375 Gy every other day for 16 days
(3 Gy Total, Cs-137)

Iron
26
Fe

650 mg Fe in High Fe diet,
45 mg Fe in Control diet for 30 days

Biospecimen Sharing



Nutrition
Ox. Damage/Minerals/Vitamins
SR Zwart/JLL Morgan/SM Smith

VIIP/Eyes
S Zanello

Pharmacology
V Wotring

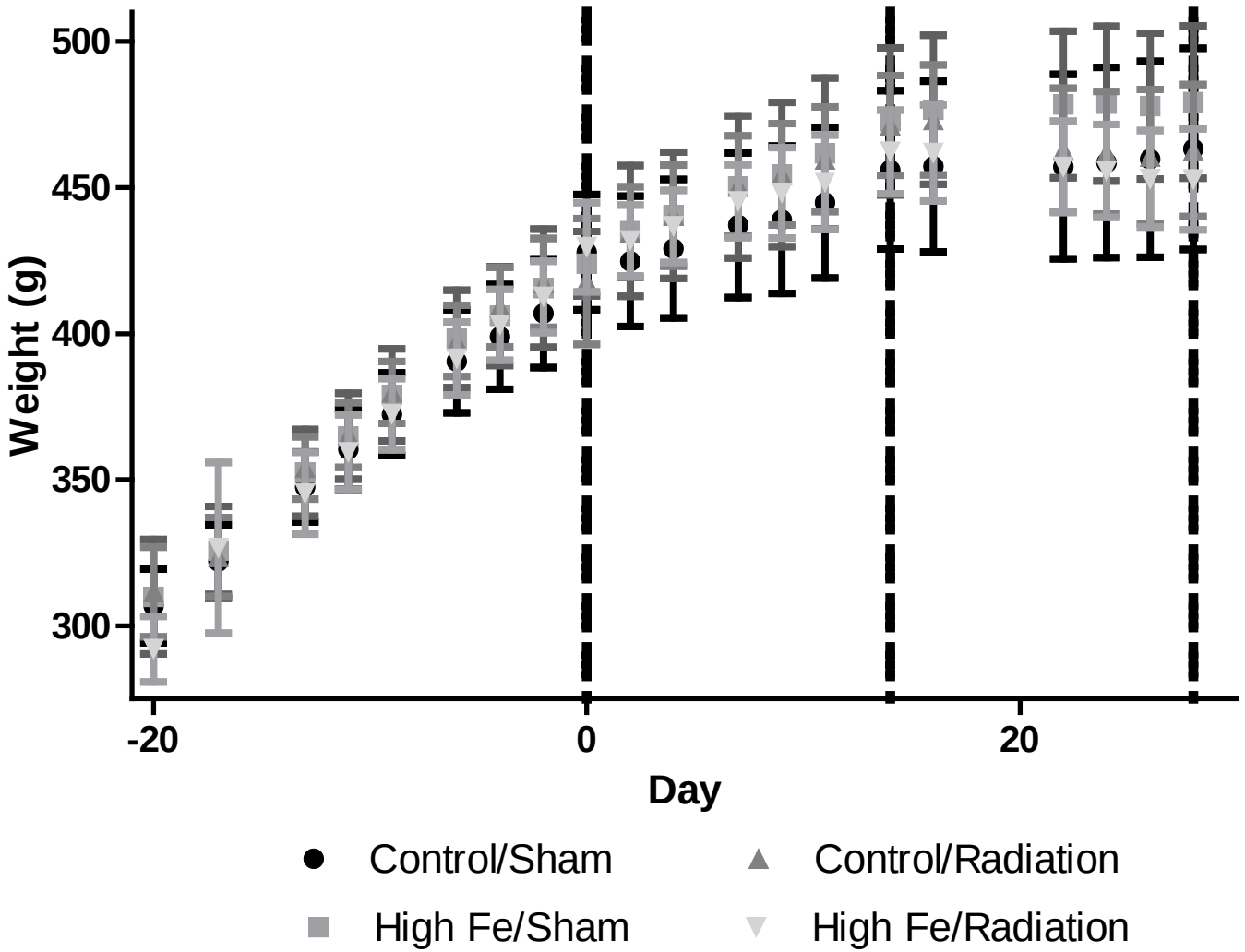
Gastrointestinal
N Turner (TAMU)

Immunology
B Crucian/C Sams

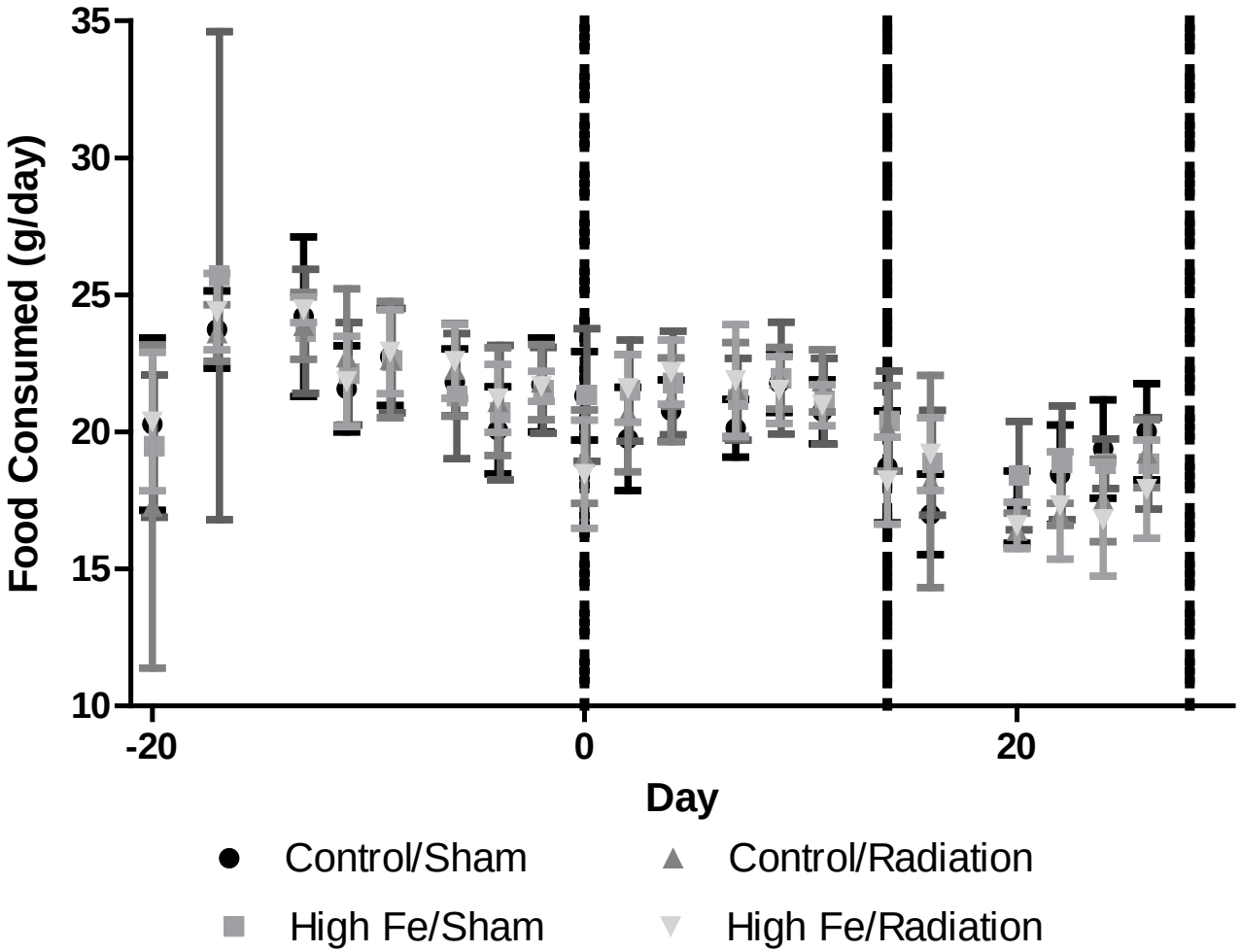
Bone
J Sibonga
S Bloomfield (TAMU)

Cardiovascular
S Platts/C Westby

Rat Weights


500
450
400
350
300
Weight (g)
-20
0
20
Day
Control/Sham
Control/Radiation
High Fe/Sham
High Fe/Radiation

Food Consumption



Nutrition Analyses on Rat Samples

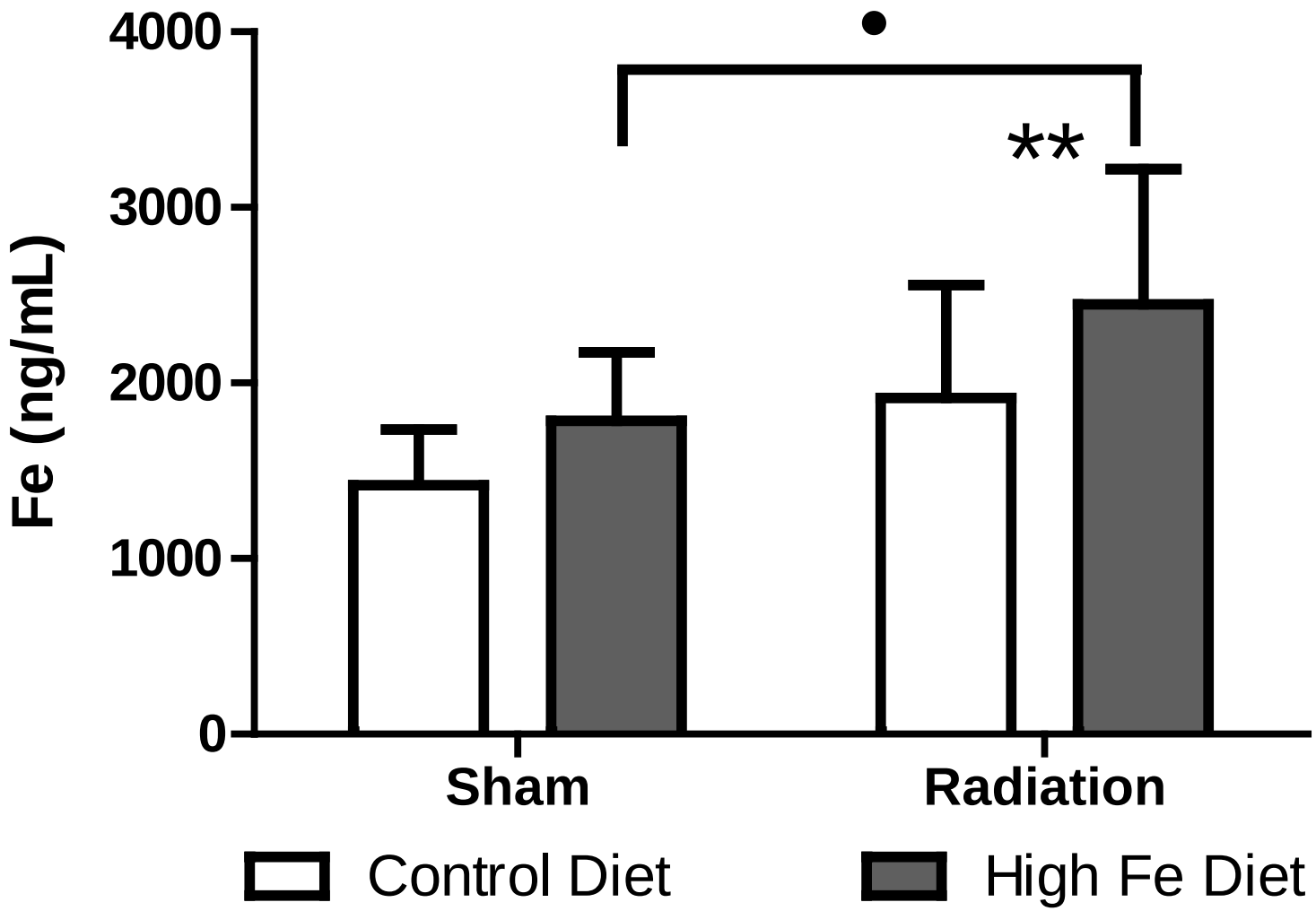
Blood

- Alanine Transaminase (ALT)
- Aspartate Aminotransferase (AST)
- RBC Folate
- Catalase
- Ferritin
- RBC Folate
- Glutathione, Oxidised & Reduced.
- RBC Glutathione Peroxidase (GPX)
- Hematochrite (HCT)
- Heme
- Hepcidin
- Hemoglobin
- NTX
- Oxidized Low density Lipids
- Superoxide Dismutase
- Total Fe, Cu, Zn, & Se
- Total antioxidant Capacity
- Transferrin
- 8OhDG
- CRP

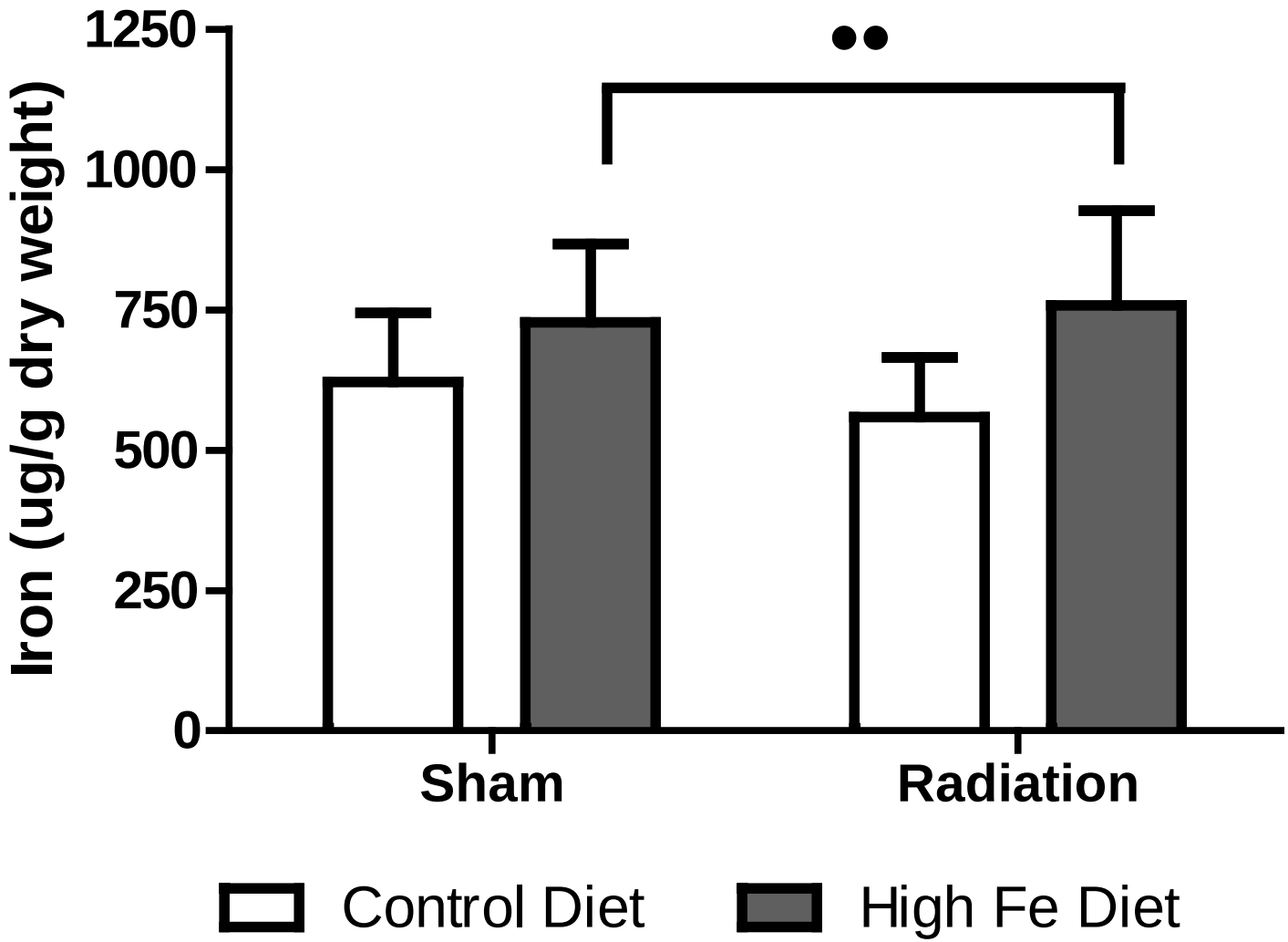
Liver

- Catalase
- Total Fe, Cu, Zn, & Se
- Glutathione Peroxidase (GPX)
- Superoxide Dismutase (SOD)
- Total Antioxidant Capacity (TAC)

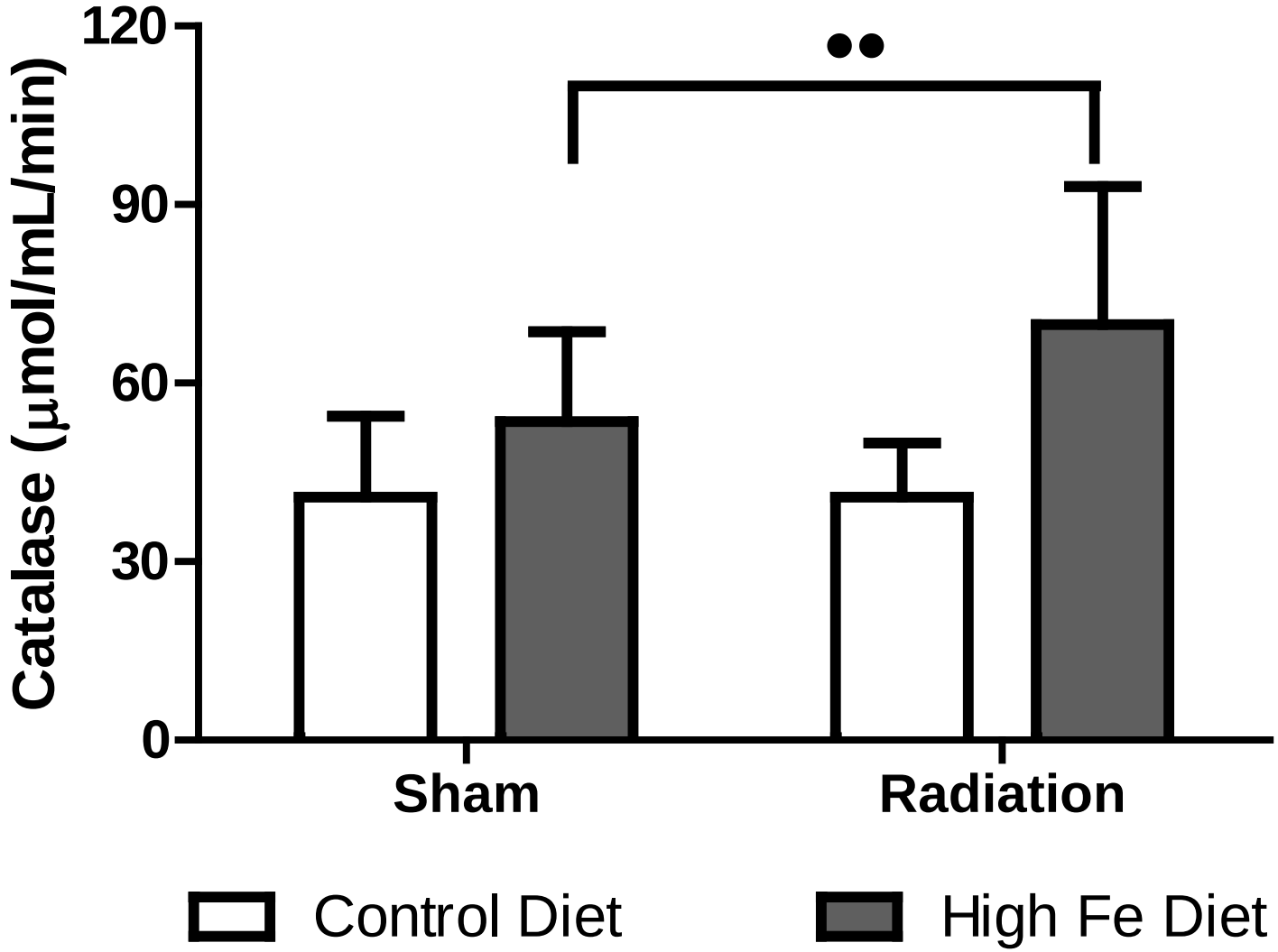
Serum Iron


4000
3000
2000
1000
0
Fe (ng/mL)
•
**
Sham
Radiation
Control Diet
High Fe Diet

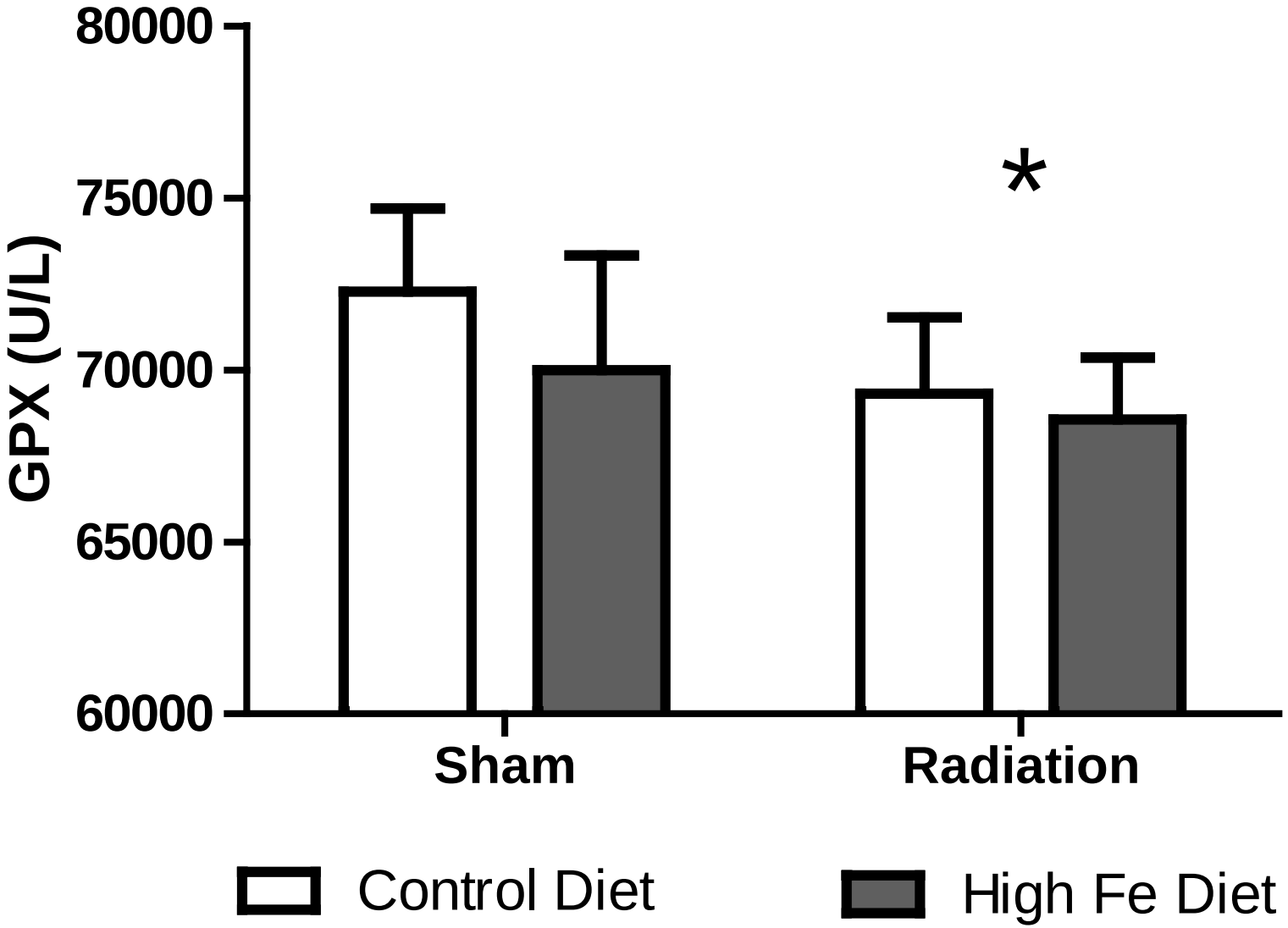
Liver Iron


1250
1000
750
500
250
0
Iron (ug/g dry weight)
••
Sham
Radiation
Control Diet
High Fe Diet

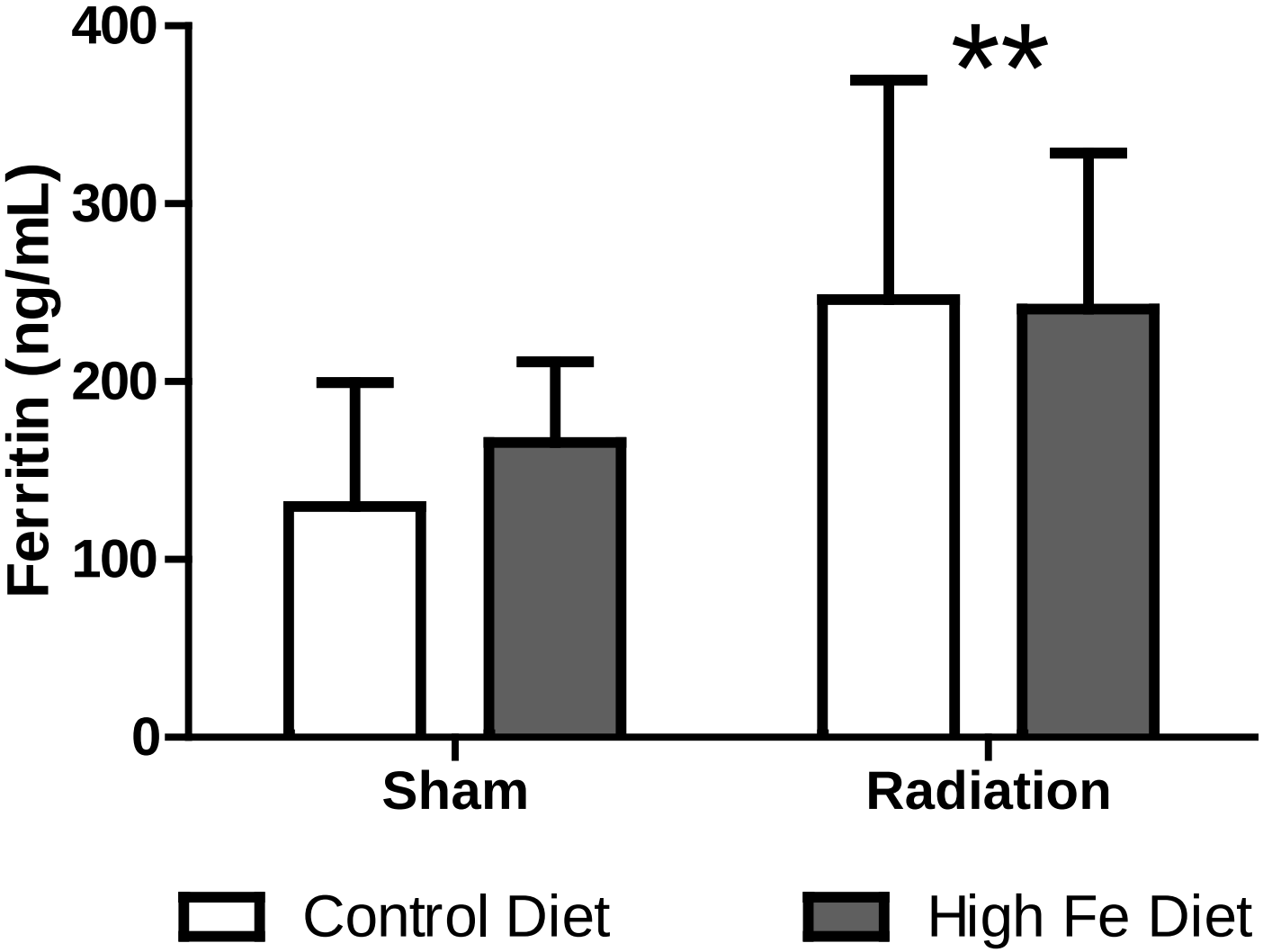
Serum Catalase



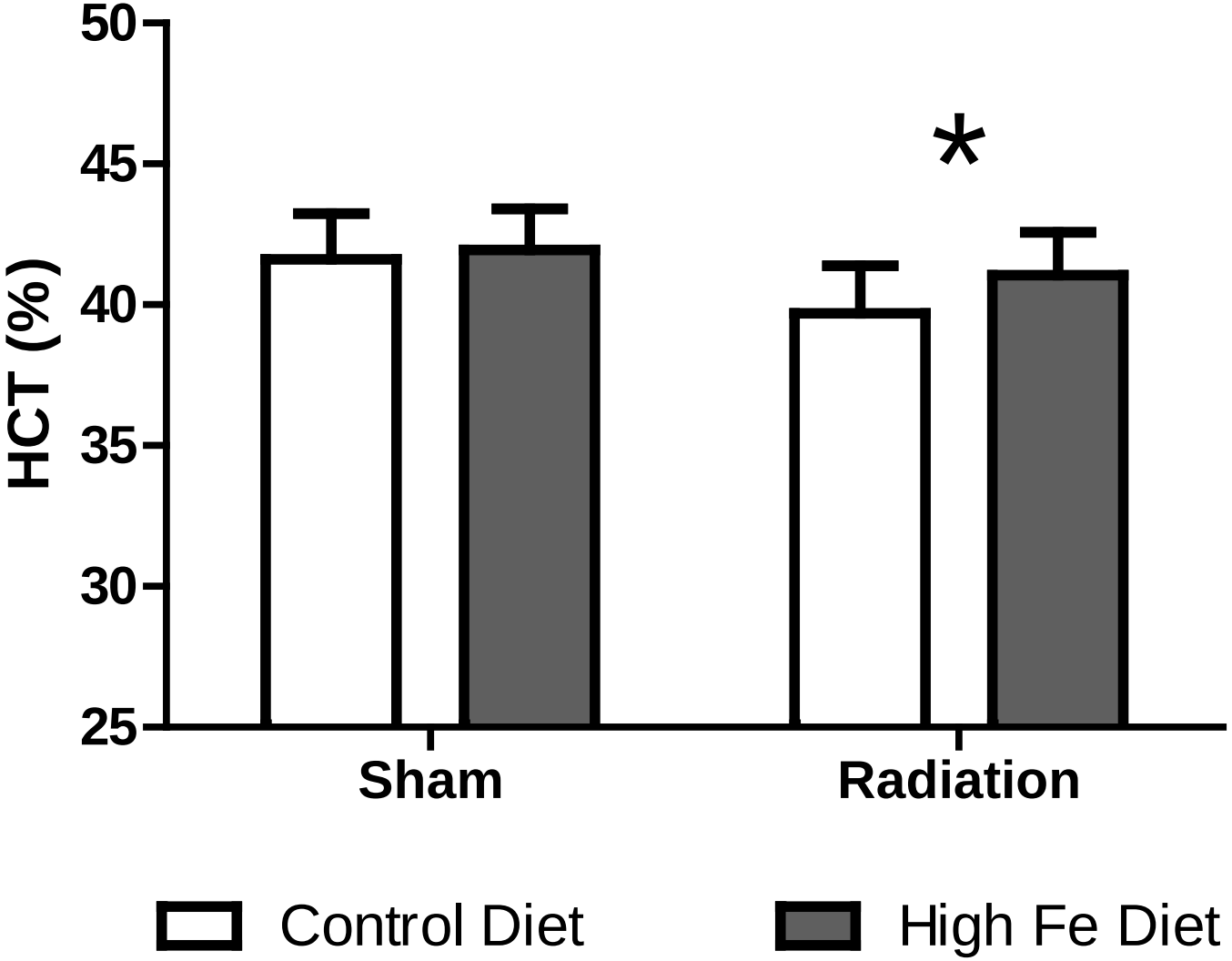
RBC GPX


80000
75000
70000
65000
60000
GPX (U/L)
*
Sham
Radiation
Control Diet
High Fe Diet

Serum Ferritin



Red Blood Cell Volume



Conclusions

- Red blood cell cycle is disrupted by radiation further increasing the TBI load
- Markers of oxidative stress are increased in serum and liver as a result of both radiation and high iron
- Other talks today will discuss the further biological implications of this oxidative stress.

STS-135 Mice

Flight Controls

Obtained samples from **7** flight mice

Give Calcein to label bone

Ground Controls

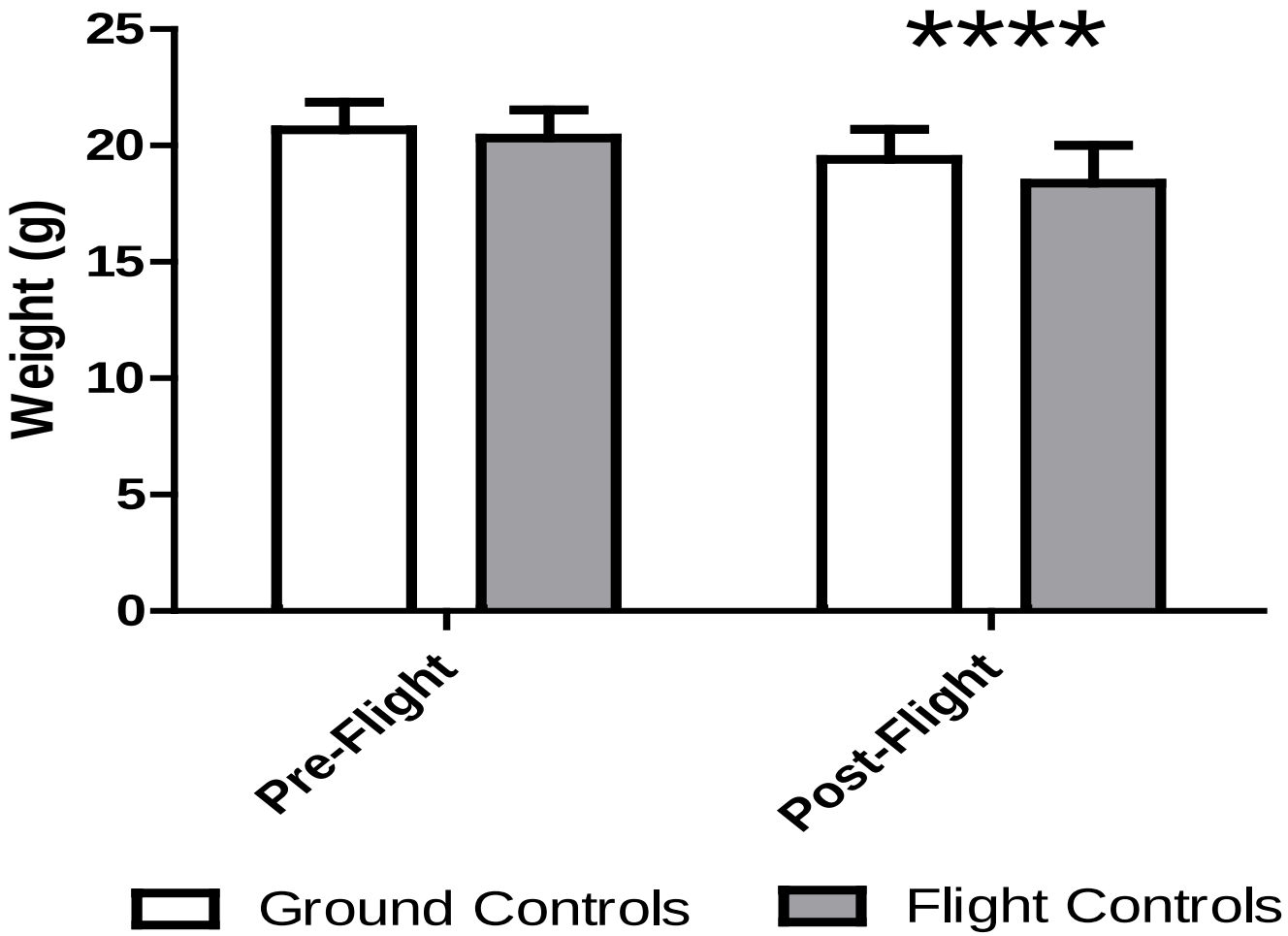
Obtained samples from **15** ground controls

Aged matched to Flight Mice

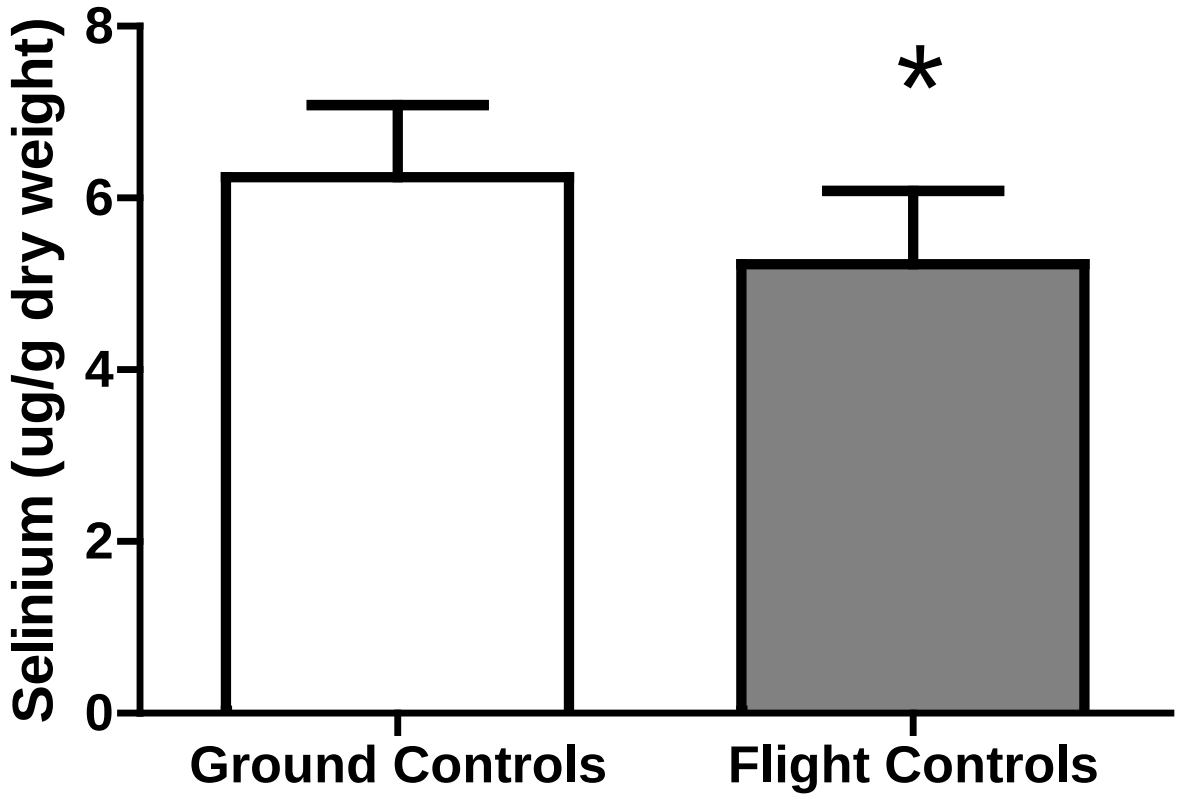
Give Calcein to label bone

Baseline Controls

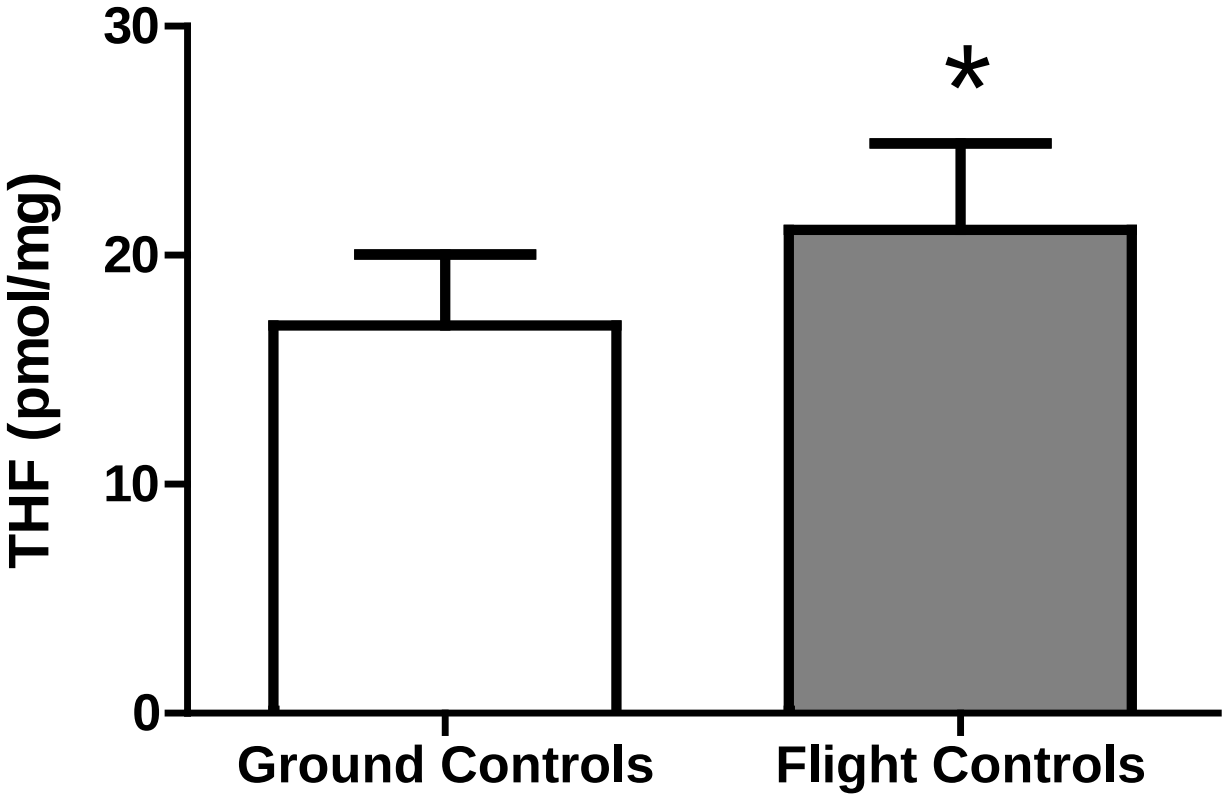
Mouse Weight



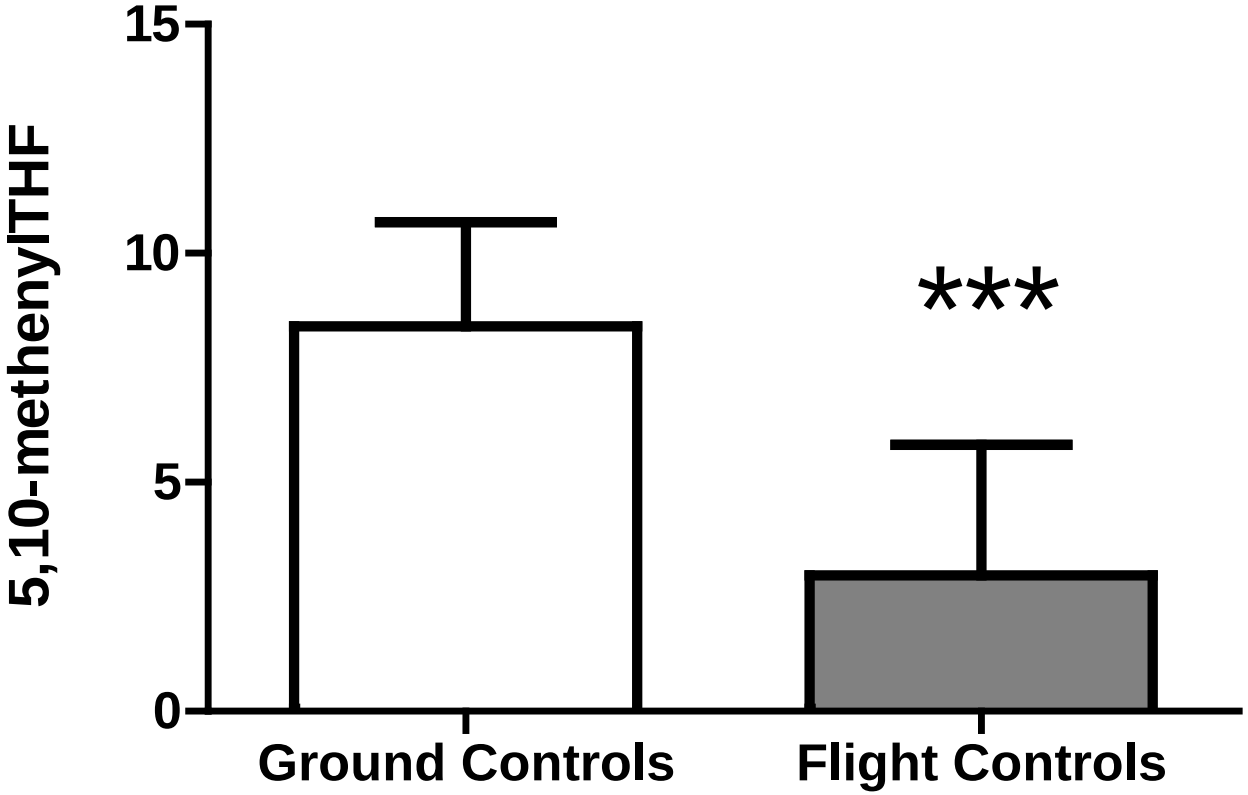
Liver Selenium



Liver Folate Metabolites



Liver Folate Metabolites



Nutrition Analyses on Mouse Samples

Liver

- Catalase
- Total Fe, Cu, Zn, & Se
- Glutathione Peroxidase (GPX)
- Superoxide Dismutase (SOD)
- Total Antioxidant Capacity (TAC)
- Folate metabolites

Conclusions

- Folate metabolism is significantly altered as a result of spaceflight
- No markers of oxidative stress are present in the liver

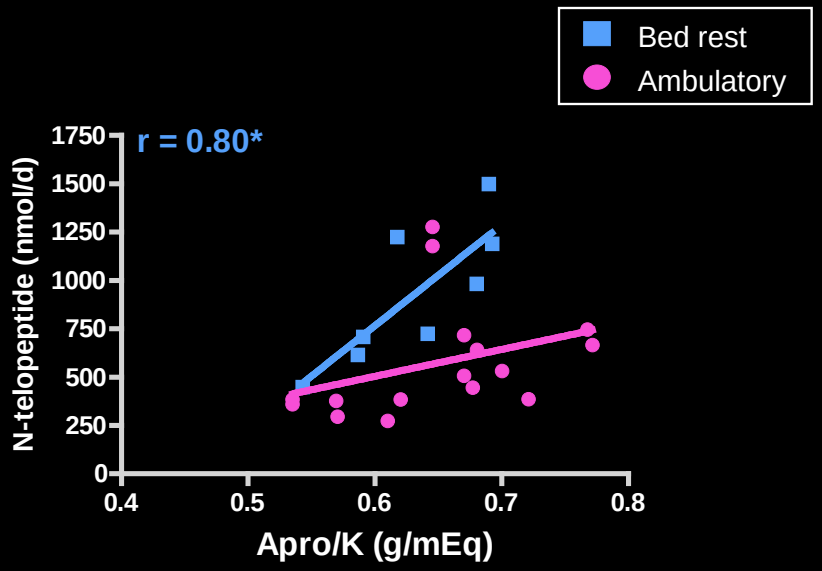
Preliminary data from the Pro K experiment:

Urinary Acid Excretion can Predict Changes in Bone Metabolism During Space Flight

SR Zwart, M Heer, L Shackelford, SM Smith

Background

- Excess protein: beneficial or harmful to bone?
  - Oxidation of excess protein yields H^+ corresponding to H_2SO_4
- Other factors
  - Calcium
  - Base-components
  - Type of protein

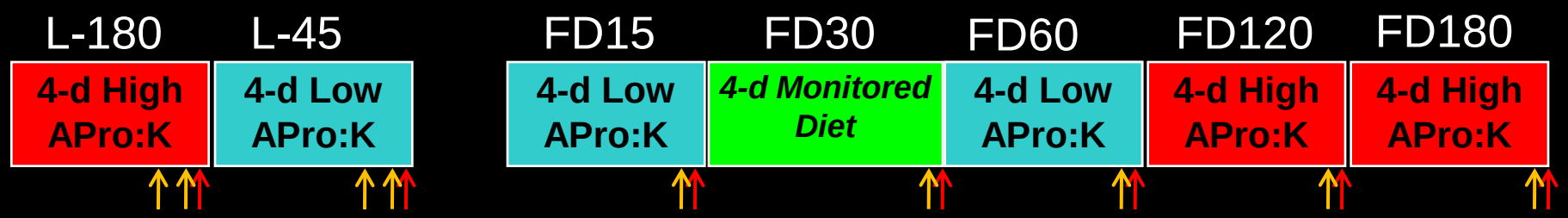


Pro K Objective

- To determine if altering the ratio of acid (animal protein) to base (potassium) precursors in the diet is associated with changes in ***bone metabolism*** during spaceflight.

Methods

- Prescribe 4-d controlled diets twice before and 4 times during flight
  - High Apro/K: 1.0-1.3 g/mEq
  - Low Apro/K: 0.3-0.6 g/mEq



- Blood and urine samples were collected at the end of each session

The following data are *preliminary*, based on n = 5

Net Acid Excretion

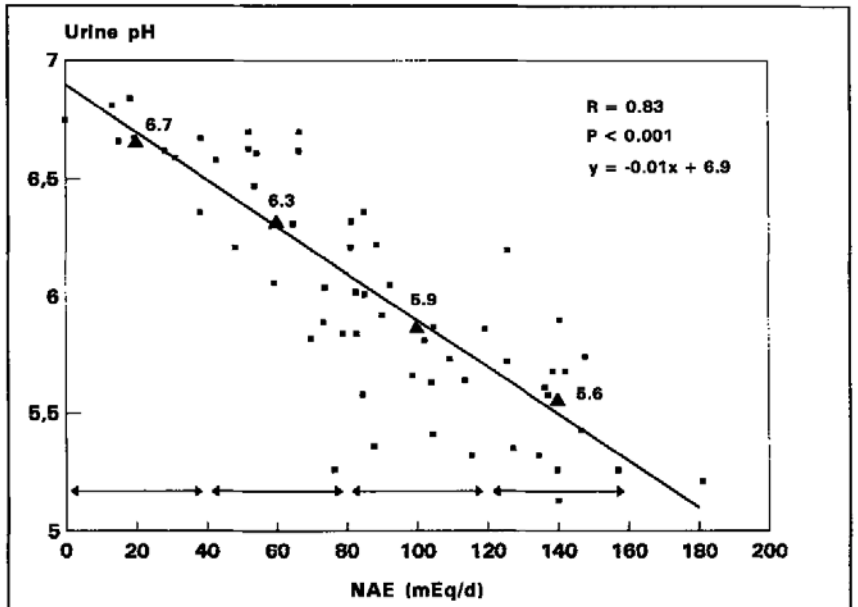
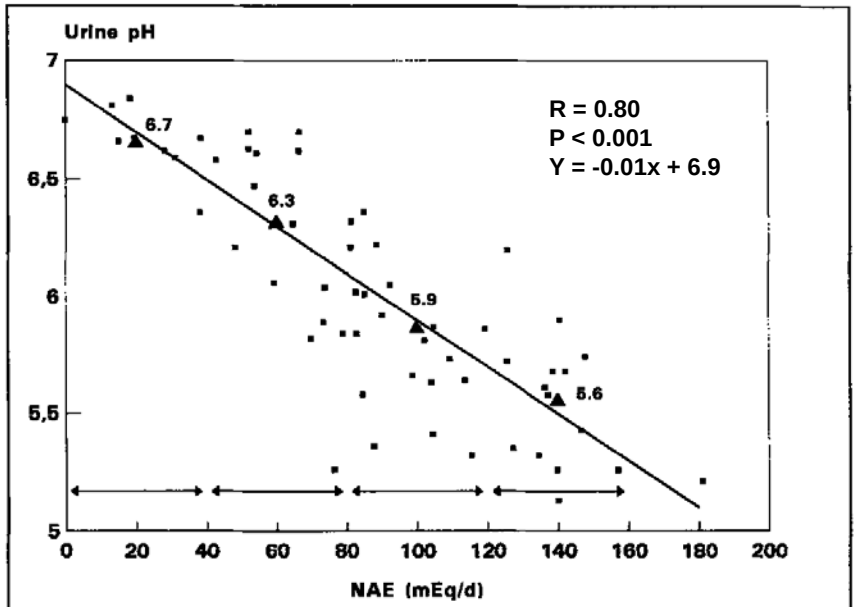


FIG 2. Association between urine pH and renal net acid excretion (NAE) in healthy males (adolescents and adults; n=60) consuming various normal mixed diets. The values above the triangles represent the urine pH means for the respective NAE intervals (each covering 40 mEq) indicated by arrows.

(Remer & Manz 1995)

NAE= (S + P + Cl + OA) – (Na + K + Ca + Mg)

Net Acid Excretion



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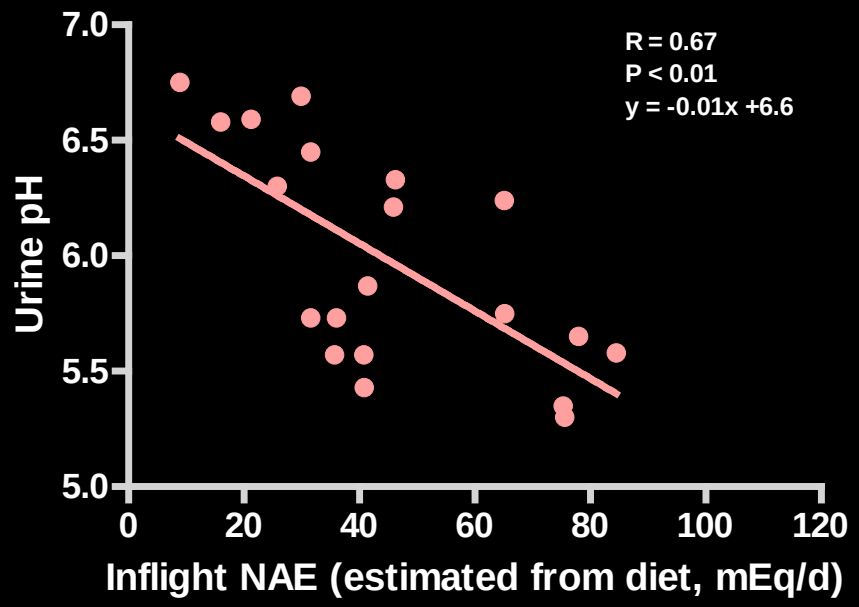
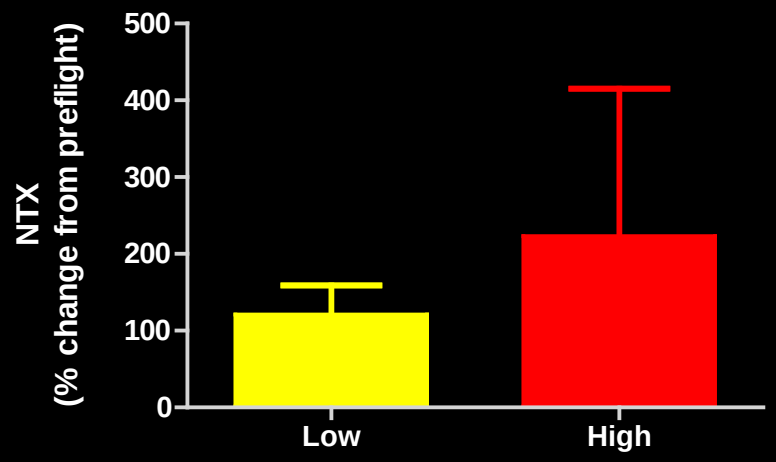
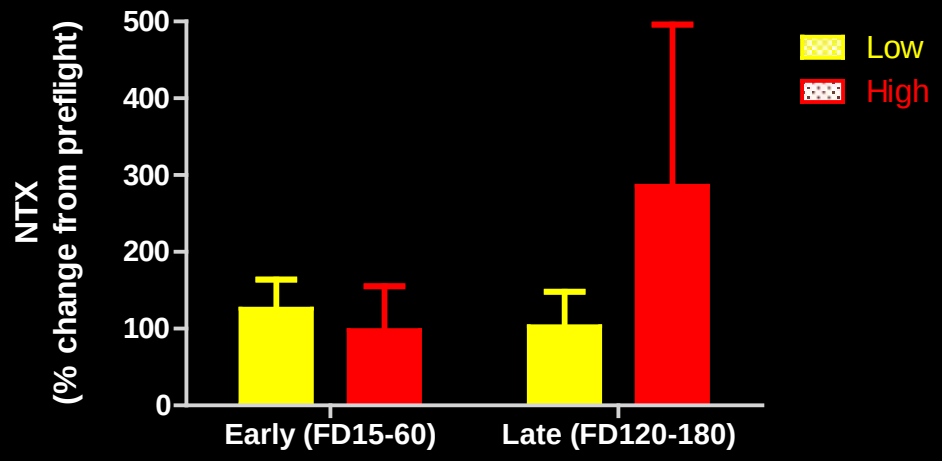


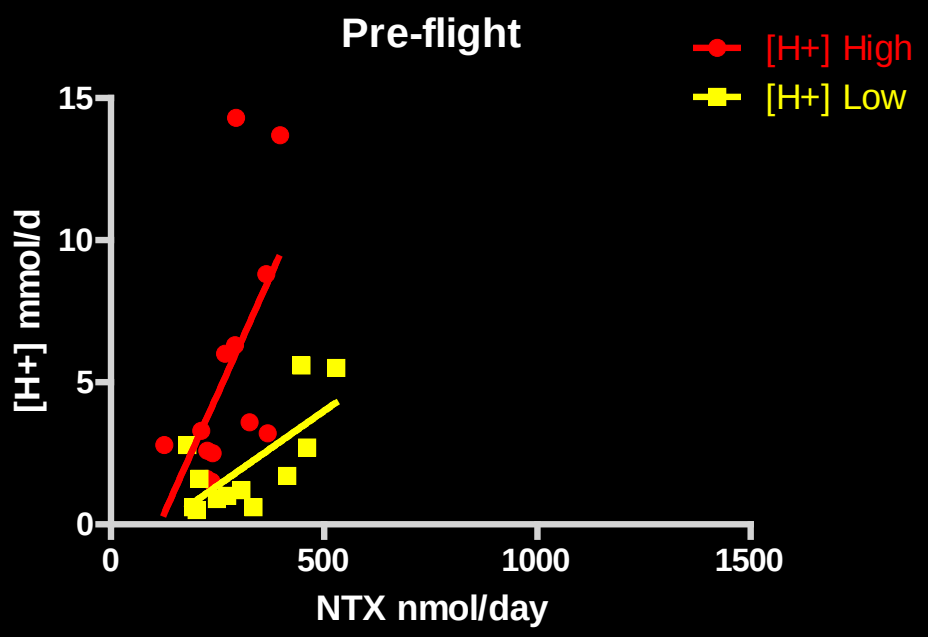
NAE= (S + P + Cl + OA) – (Na + K + Ca + Mg)

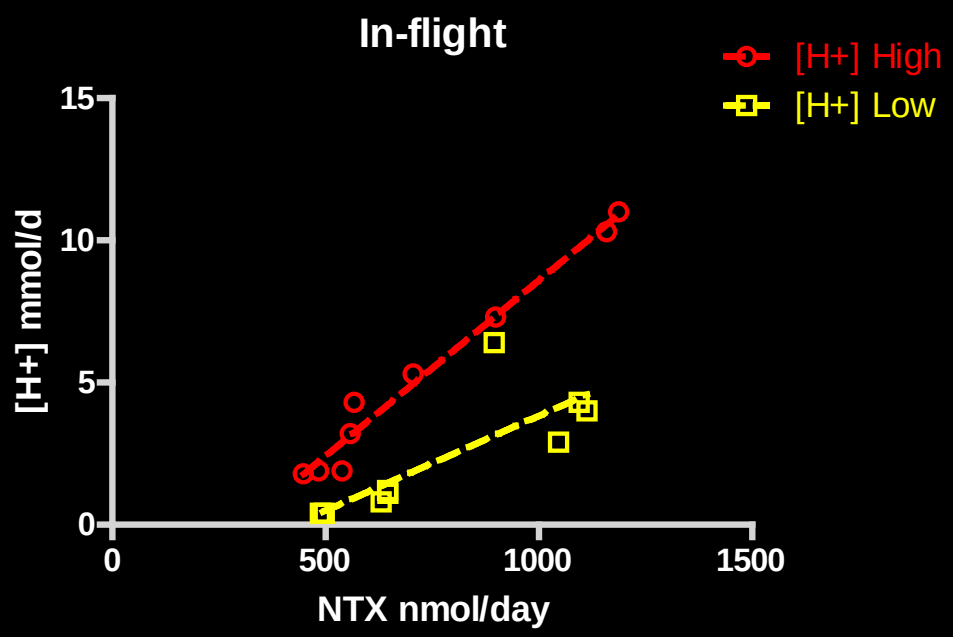
Bone Resorption (NTX)





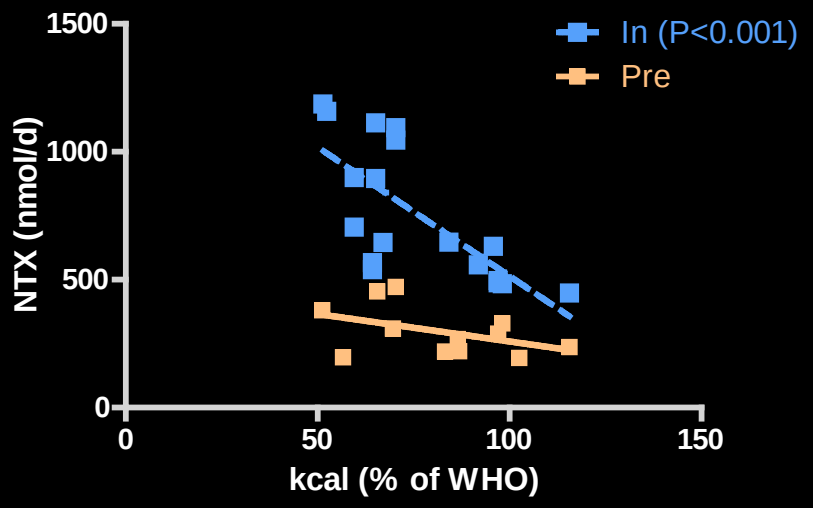
Bone Resorption (NTX)

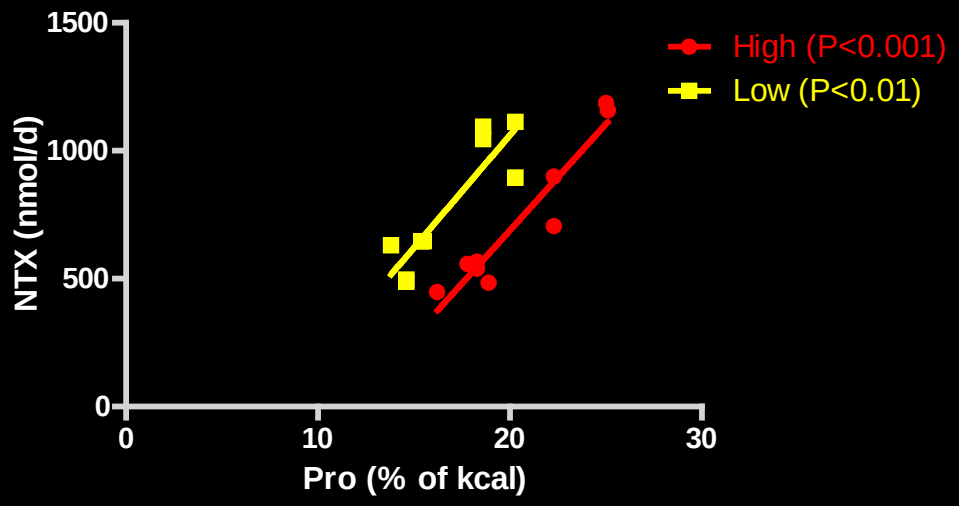




Confounding Factors?

- Energy (i.e., kcal)
- Protein (% of kcal)
- Exercise
- Medications
- Other?





Forward Work

- 2 Pro K subjects currently on ISS
- Additional Pro K subjects scheduled to fly in next 2 years
- Frozen sample return pending commercial launch vehicles (late 2012/TBD)