Integration of Salivary Bioscience into Behavioral, Health, and Sports Sciences:

How Biomarkers in Saliva can Benefit Your Research Program

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Disclosure Statement

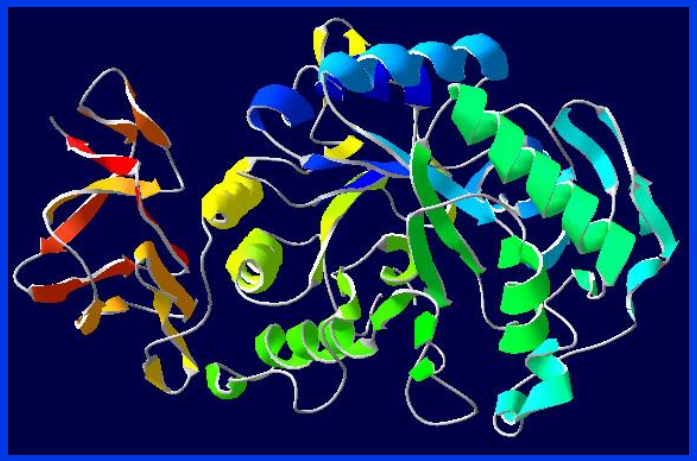
In the interest of full disclosure, Douglas A. Granger is the founder (or co-founder), serves as the Chief Scientific and Strategy Advisor, and holds equity in Salimetrics LLC and Salivabio LLC.

These relationships are managed by the policies of the Conflict of Interest Committee at the Johns Hopkins University School of Medicine

Overarching Assumptions

Effects of context on development moderated through individual differences in stress responsive biological systems

Biology and behavior have reciprocal effects and expression of bio-behavioral relationships is dependent on context



Probability that individual differences in biological reactivity and regulation linked to outcomes of interest highest when studied in meaningful social contexts

Biological systems are networked and multi-system measurement of stress response is critical

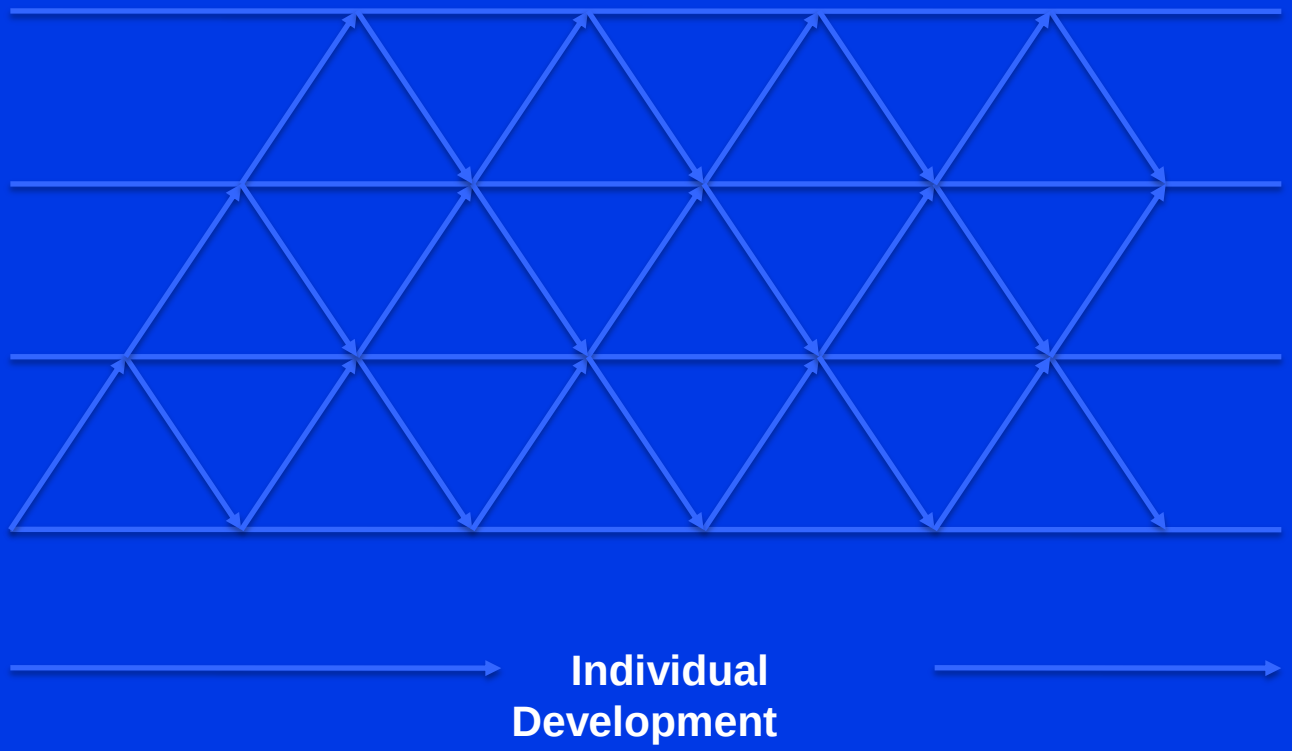
Perturbing the organism: The biology of stressful experience

Environmental Demands
(physical, social, cultural)

Behavioral Surface
(emotion regulation, coping, flight-fight, tend-befriend)

Fast Acting—Physiological Processes
(neural, HPA, ANS activity)

Slow Acting—Physiological Processes
(genetic activity)

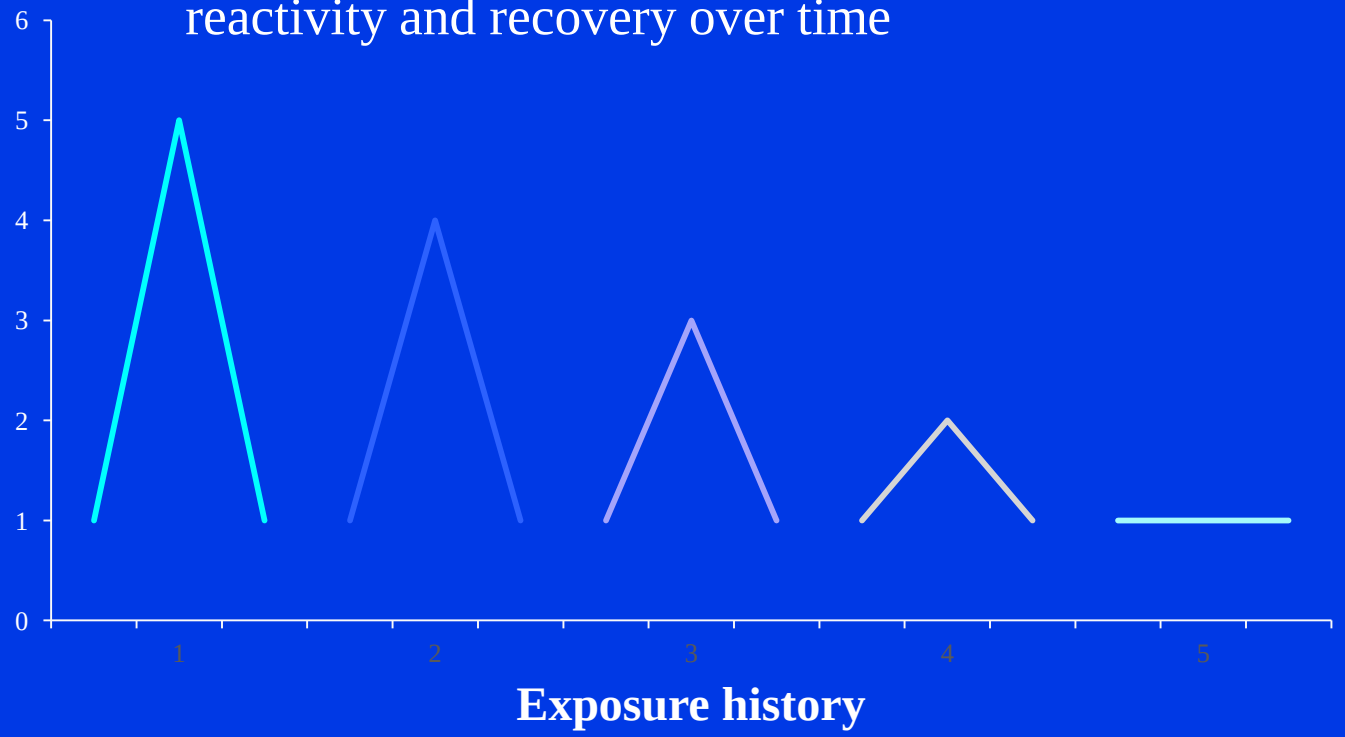


Biological Susceptibility to Context, Adaptive Calibration

Weiner (1992) Gottlieb (1992) Boyce and Ellis (2005)

Intra-individual patterns of stress-reactivity

Normative-adaptive pattern assumes habituation of reactivity and recovery over time



Time

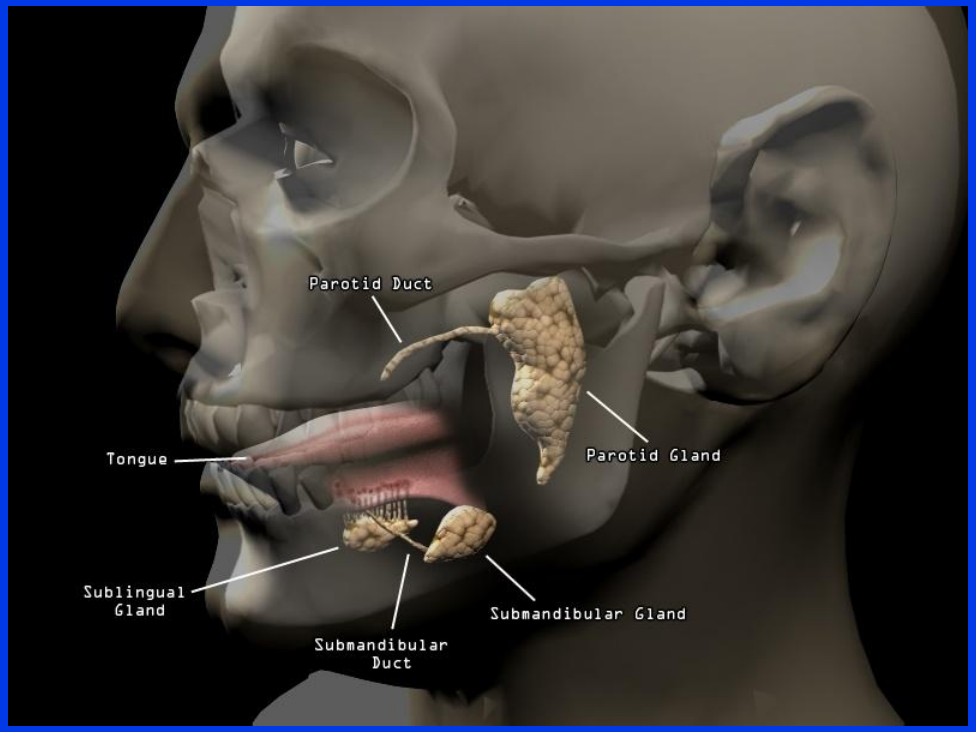
McEwen

Advantages of Oral Fluid as Research Specimen

"Minimally Invasive"	Considered "acceptable and non-invasive" by research participants Collection is quick, non-painful, uncomplicated
"Safety"	Reduces transmission of infectious disease by eliminating the potential for accidental needle sticks CDC does not consider saliva a class II Biohazard unless visibly contaminated with blood
"Self-collection"	Allows for community- and home-based collection Enables specimen collection in special populations
"Economics"	Eliminates the need for a health care intermediary (e.g., phlebotomist, nurse). Resources for collection and processing samples are low cost and available
"Accuracy"	Salivary levels of many analytes represent the "free unbound fraction" or biological active fraction in the general circulation
"Ecological Validity"	Enables biological reactivity and regulation to be monitored in everyday social world
" Multiple Participants"	Enables samples to be collected from groups of individual participants simultaneously in real time

Slavkin /Mandel/Malamud

Oral fluid subtypes that compose “Whole Saliva”



Sublingual saliva (4%)

Parotid saliva (23%)

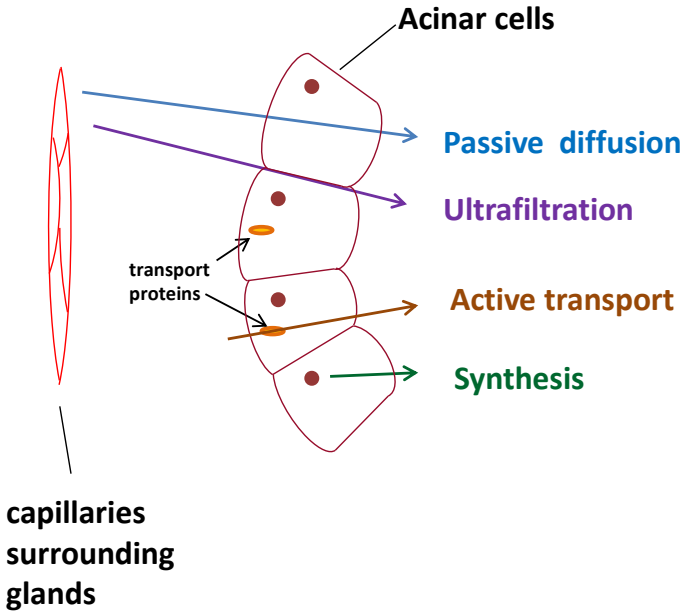
Submandibular saliva (65%)

Minor saliva glands (7%)

Cervicular fluid (1%)

Mandel

Movement of Biomarkers into Saliva

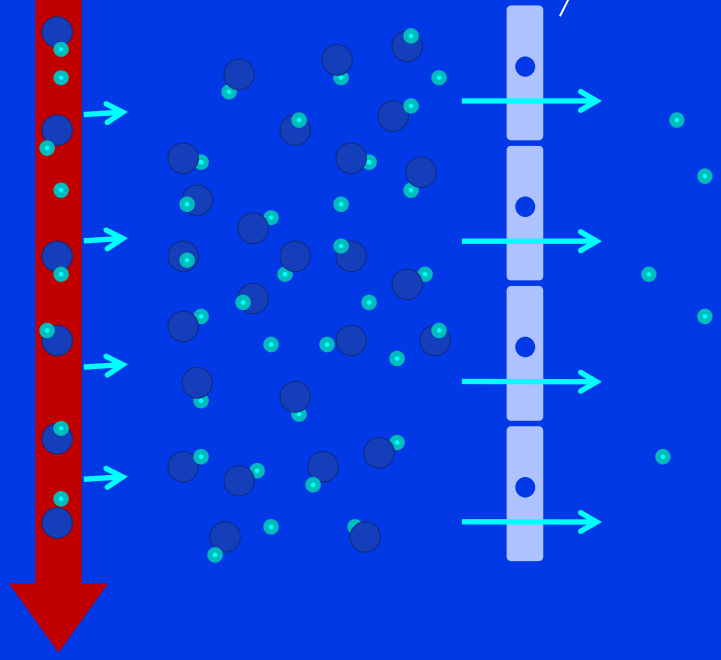


Adrenal Cortex

Cortisol is secreted into the bloodstream from the adrenal cortex. About 95% of it binds to carrier proteins (binding globulin). Only the remaining 5% is available for use by target tissues.

Numerous capillaries surround the saliva glands. Hormones and other compounds pass through the capillary walls and bathe the salivary glands.

Secretory cells of the saliva glands



Saliva

Unbound cortisol is lipid soluble and can move through the lipo-protein cell membranes of the secretory cells by passive diffusion. It is then released into the saliva.

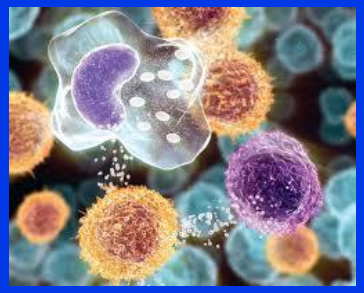
Many analytes are released locally into oral fluids and are not from blood

Knowing whether an analyte measured in oral fluid is or is not from blood is key to interpretation of the meaning of individual differences

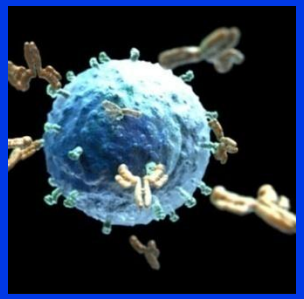
Analytes in Oral Fluid of Interest to Health Sciences

- Cortisol
- Dehydroepiandrosterone (s)
- Testosterone, androstenedione
- Estradiol, estrone, estriol
- sIgA
- Progesterone and 17-alpha-hydroxy-Progesterone
- Cotinine
- Drugs of Abuse and Environmental Chemicals
- C-Reactive Protein, Neopterin, Beta-2-microglobulin
- Melatonin, Oxytocin, NPY, VIP
- Cytokines (e.g., IL-6, TNFa, IL1b), soluble cytokine receptors (sTNF-I, -II)
- Disease specific antibodies (e,g, HIV, HSV, EBV, CMV) or antigens
- Alpha-amylase, Chromogranin A
- Metalloproteinases (MMP-8)
- Heat Shock Proteins, aldosterone
- Adiponectin, Leptin, Ghrelin
- Electrolytes
- DNA and Methylation, RNA
- Microbiome
- GFAP, Neurogranin

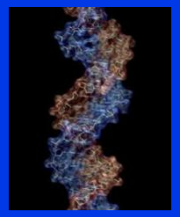
cytokines



Antibodies



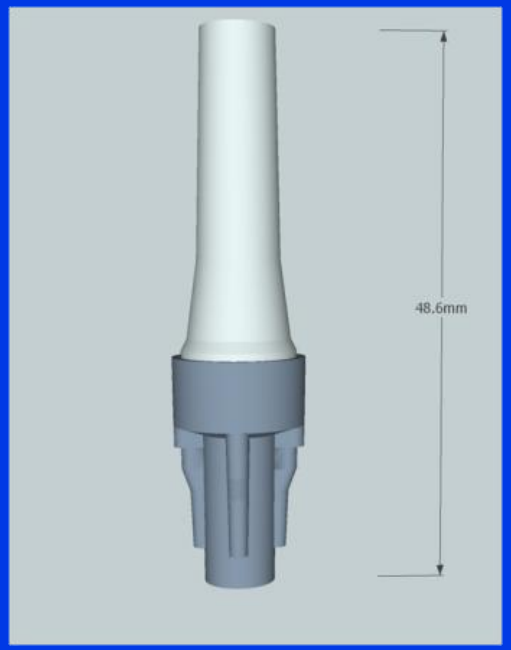
DNA

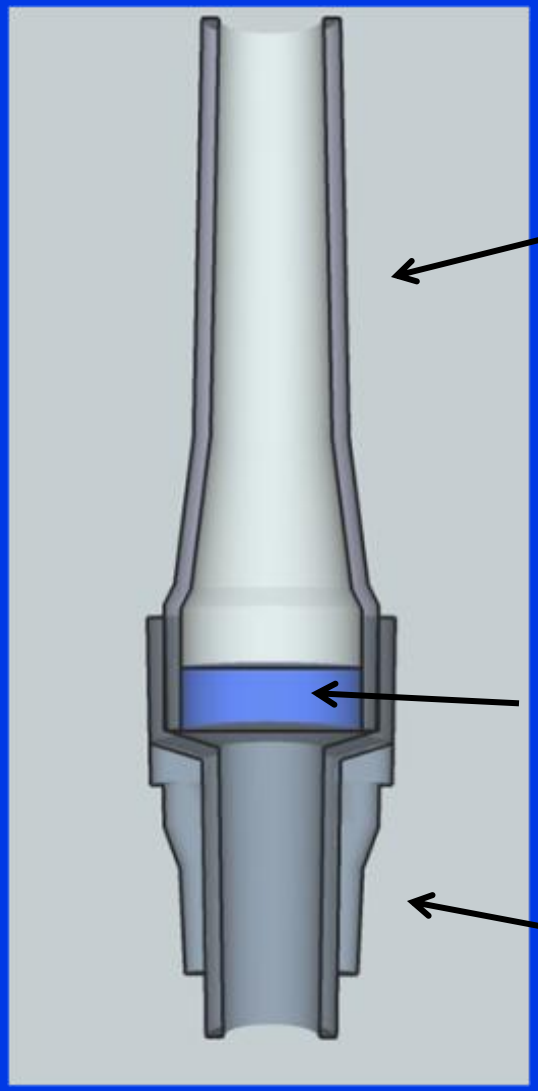


THE NATIONAL CHILDREN'S STUDY

HEALTH GROWTH ENVIRONMENT

Collection Device Aid





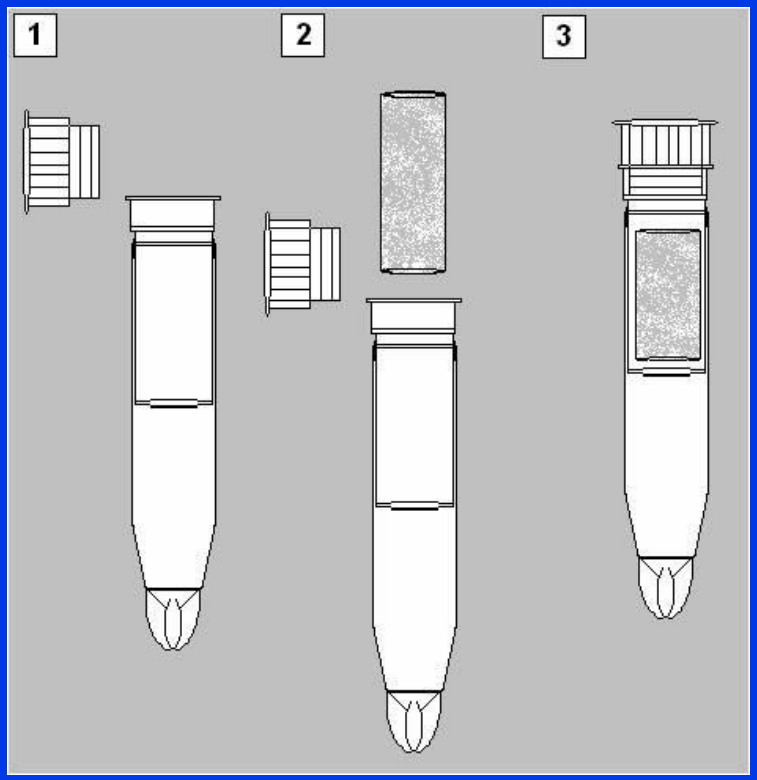
Mouth Piece and Flute

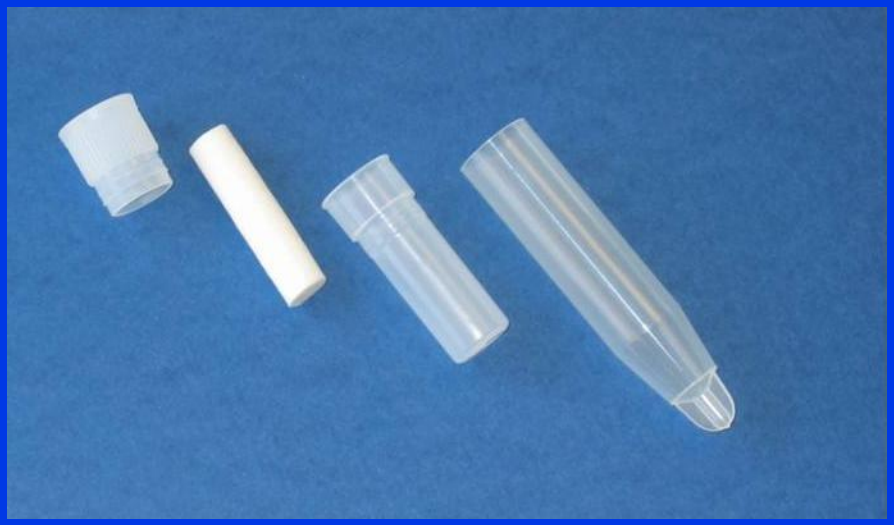
Treatment Chamber

Vented connector to storage vial

Salivette Module with Oral Swab

Easy to use
Excellent Recovery
1.5 mL capacity

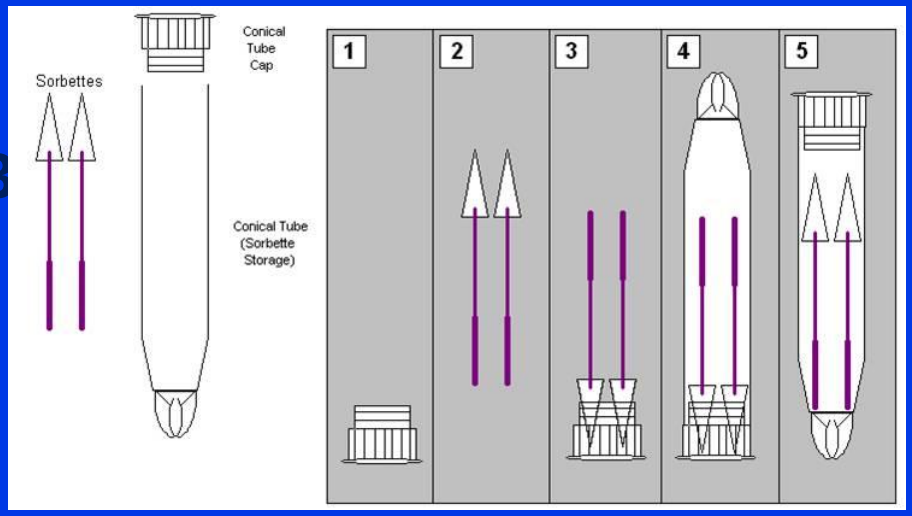




Restricted analysis potential
Small size-choking hazard
Large head space
Location of placement

BD Hydrocellulose Microsponge

Handle
Highly absorbent
Recovery of low volumes
Sleeping participant



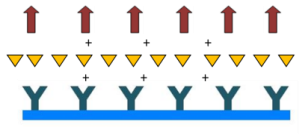
High surface area
Evaporation hazard
Use two
Restricted analysis

Harmon et al (2007). Hormones and Behavior.

Handling, Transport, Storage

- Biosafety
- Bacteria growth and specimen stability
- Freeze thaw
- Field storage
- Shipping rules and regulations
- Long term storage and archiving

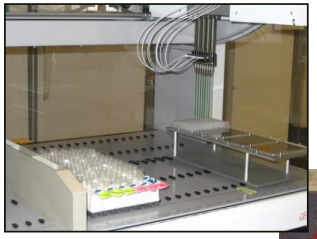
Enzyme Immunoassay Competitive Binding

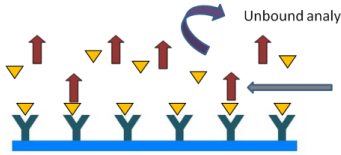


Conjugate

Standard and/or sample are added

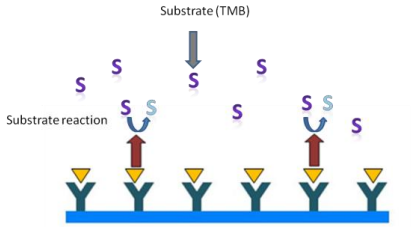
Antibody on EIA plate





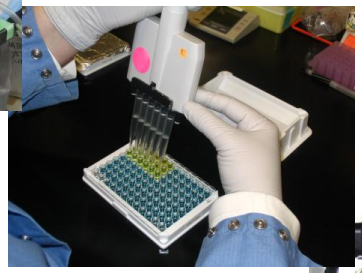
Unbound analytes are washed away

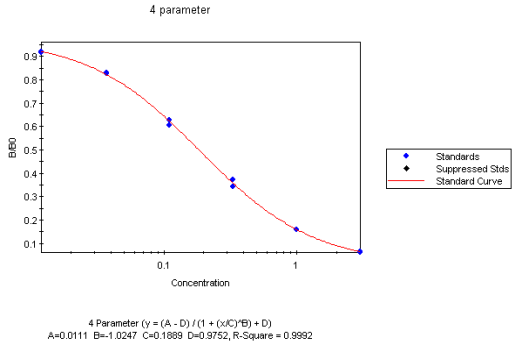
Bound analytes



Substrate (TMB)

Substrate reaction





4 parameter

B/B0

Concentration

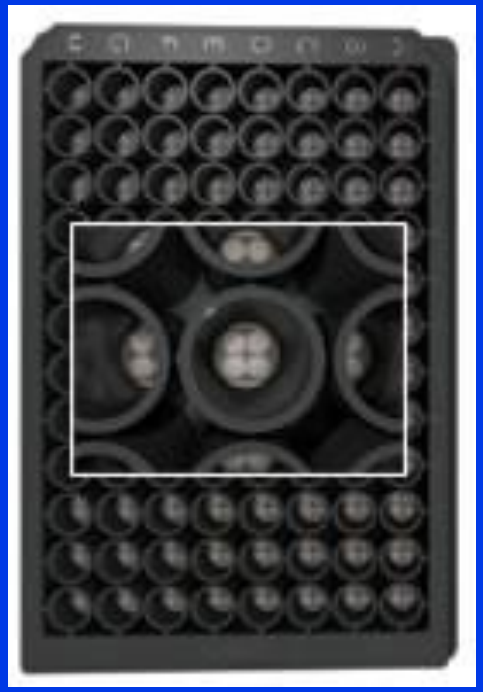
Standards

Suppressed Stds

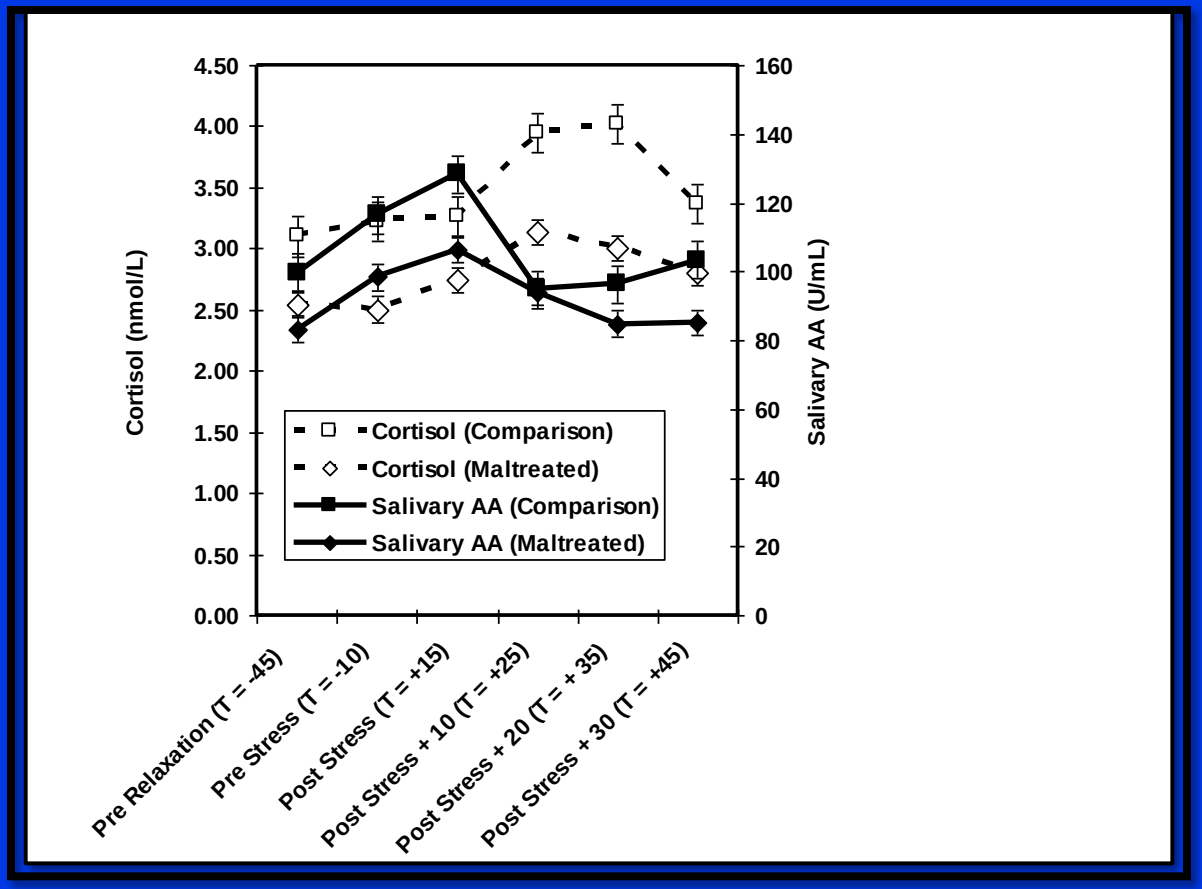
Standard Curve

4 Parameter (y = (A - D) / (1 + (x/C)^B) + D)
A=0.0111 B=-1.0247 C=0.1889 D=0.9752, R-Square = 0.9992

Multiplexing Technology



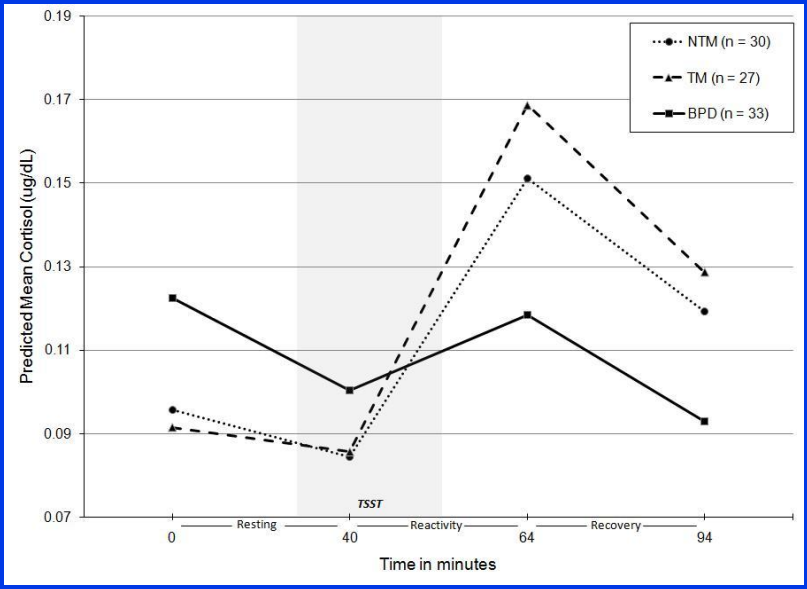
Dampened Salivary Alpha-amylase and Cortisol Reactivity to Psychosocial Stress : Maltreated versus Comparison Adolescents



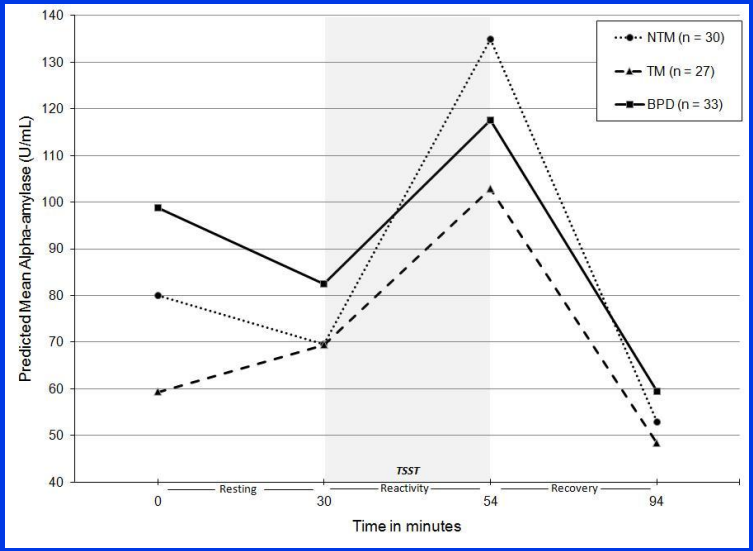
Gordis et al (2008) Psychoneuroendocrinology

Cortisol, Alpha-amylase, and subjective emotional reactivity in women with Borderline Personality Disorder (BPD)

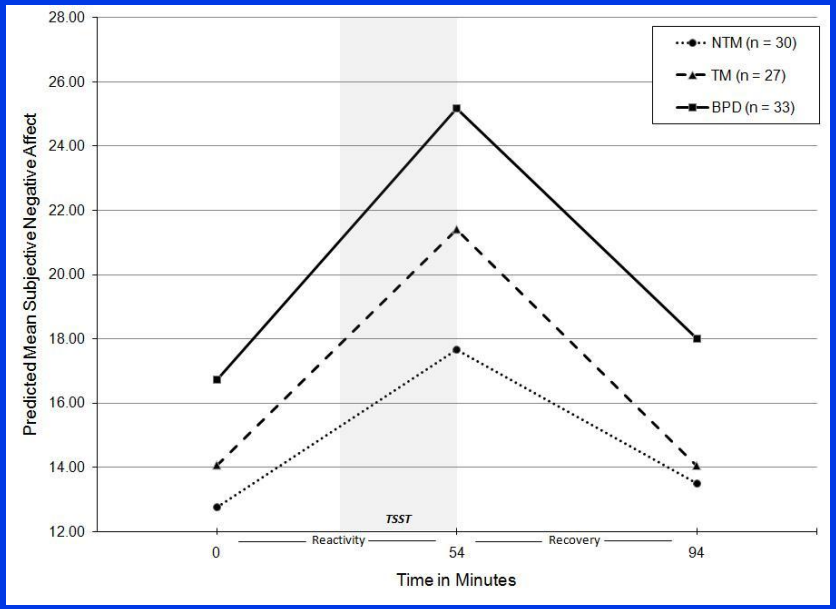
(Scott, Granger, Levy, 2011)



. The BPD group showed attenuated stress-related cortisol reactivity as compared to both NTM and TM groups.

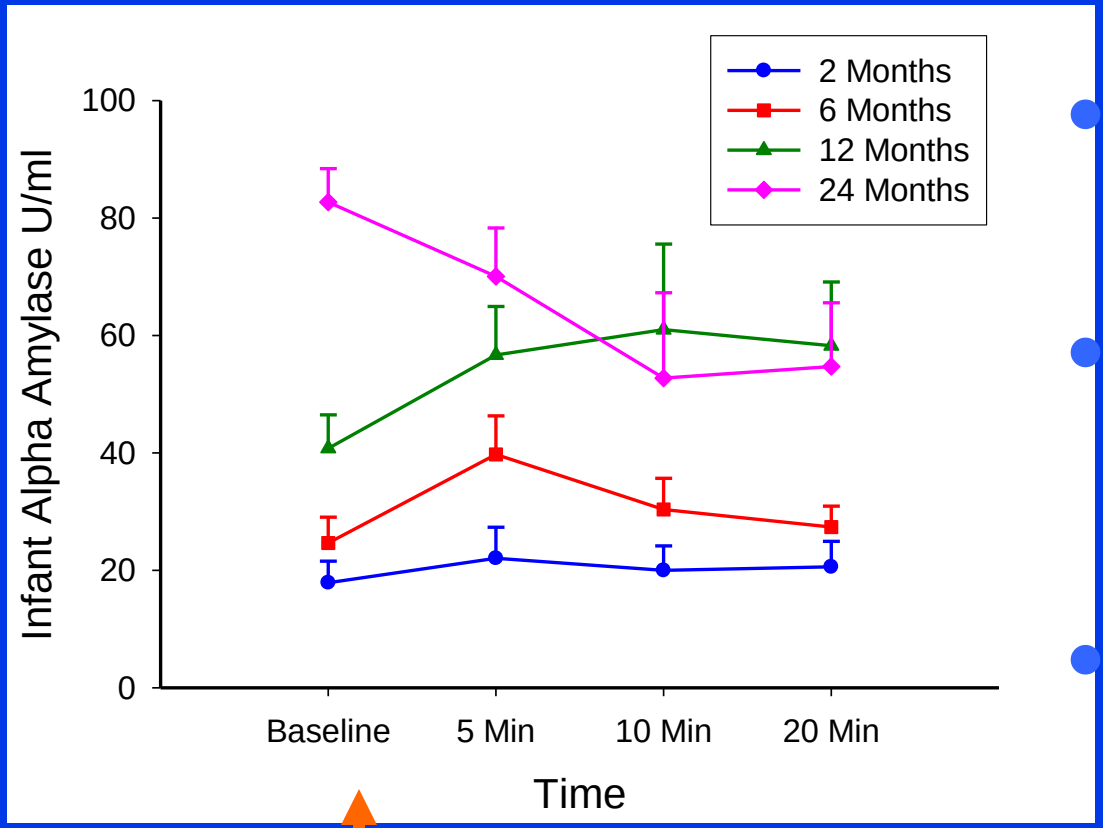


Both the BPD and TM groups demonstrated attenuated stress-related sAA reactivity as compared to the NTM group.



The BPD group had higher average baseline and overall average NA than both NTM and TM groups.

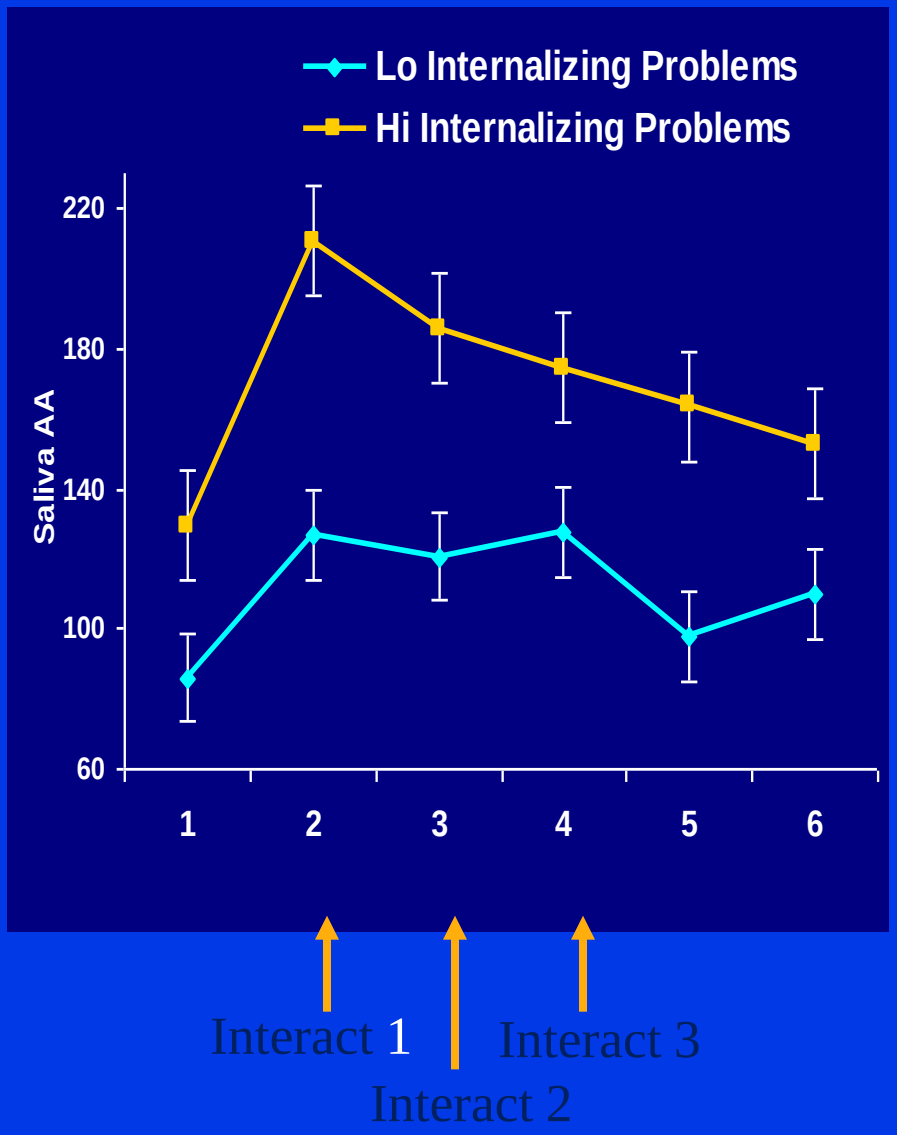
Developmental differences in sAA Response to Inoculation Stress



- 2-month old infants did not show an sAA response
- 6 and 12-month old infants displayed a significant sAA increase.
- 24-month old infants displayed an anticipatory rise and decrease in sAA

Davis & Granger (2009). *Psychoneuroendocrinology, 34, 795-804*

Internalizing problem behavior and sAA reactivity to social exclusion task



Allwood et al., (2011). Biological Psychology, 88, 57-64

Maternal Engagement in Early Infancy Predicts Children's Cortisol Reactivity at 15 months

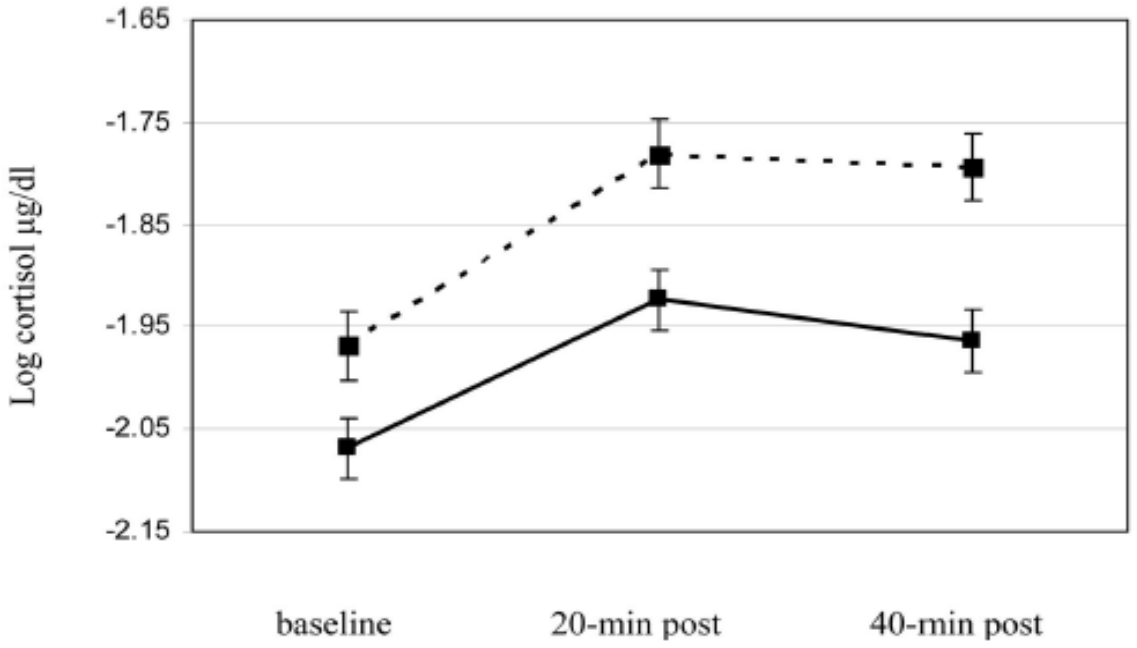


Figure 2. Cortisol reactivity and regulation in response to emotion challenge in the toddler period as a function of maternal engagement in infancy. Dashed line = low maternal engagement; solid line = high maternal engagement as defined by median split; post = post–peak arousal. Error bars represent the sampling error of the mean.

Blair et al, (2008) Developmental Psychology, 44, 1095-1109

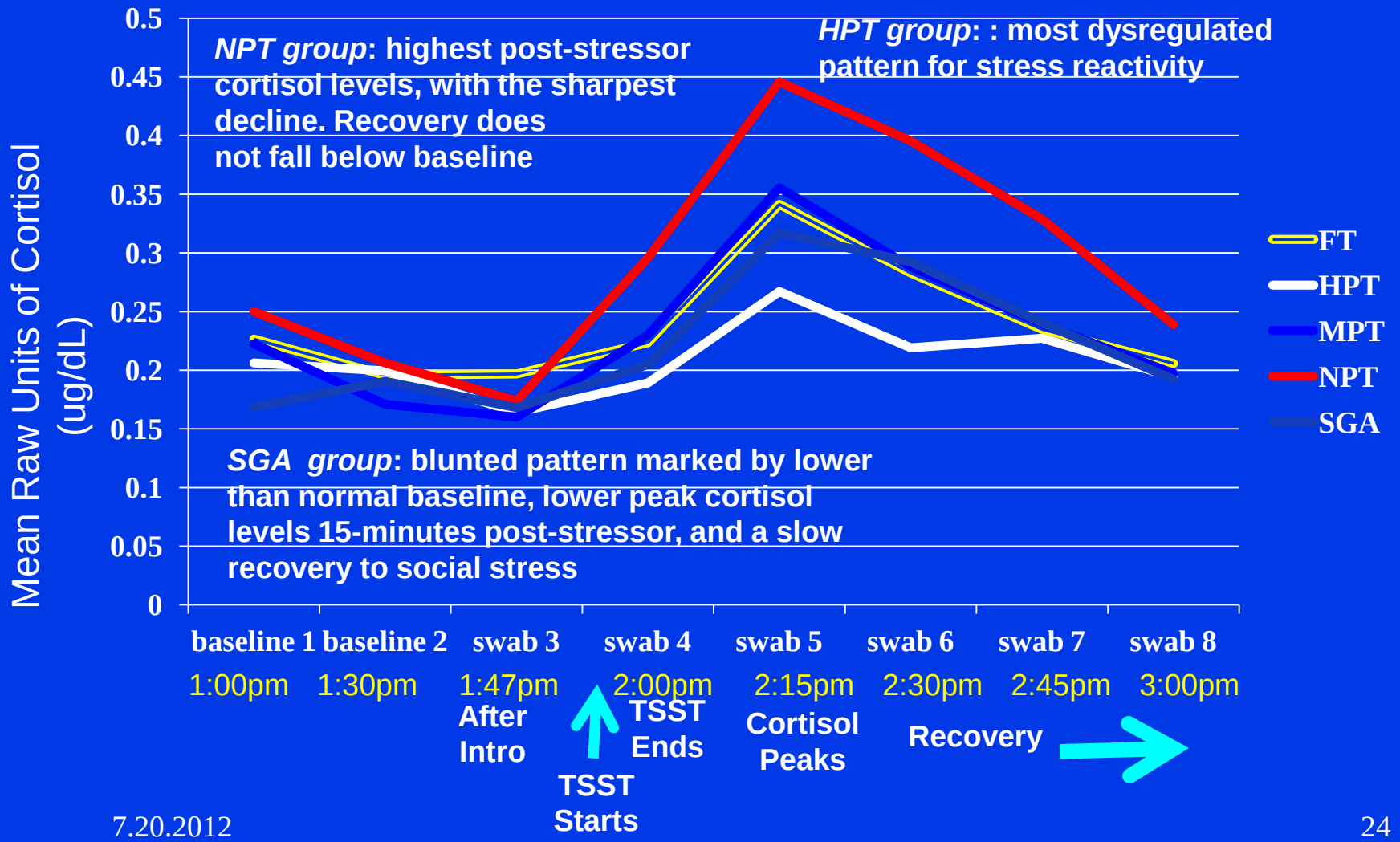
Effects of early adversity on stress reactivity in adulthood

Birth Group	*Neonatal Criteria*
Full Term	Full Term; medically & neurologically healthy
Healthy Preterm	Premature no medical/neurological complications
Medical Preterm	Premature neonatal medical illness (BPD, RDS, NEC, sepsis)
Neurological Preterm	Premature neonatal neurological illness (Grade III & IV IVH, meningitis, shunted hydrocephalus)
Small for Gestational Age Preterm	Premature birth weight for gestational age < 10^{th} percentile

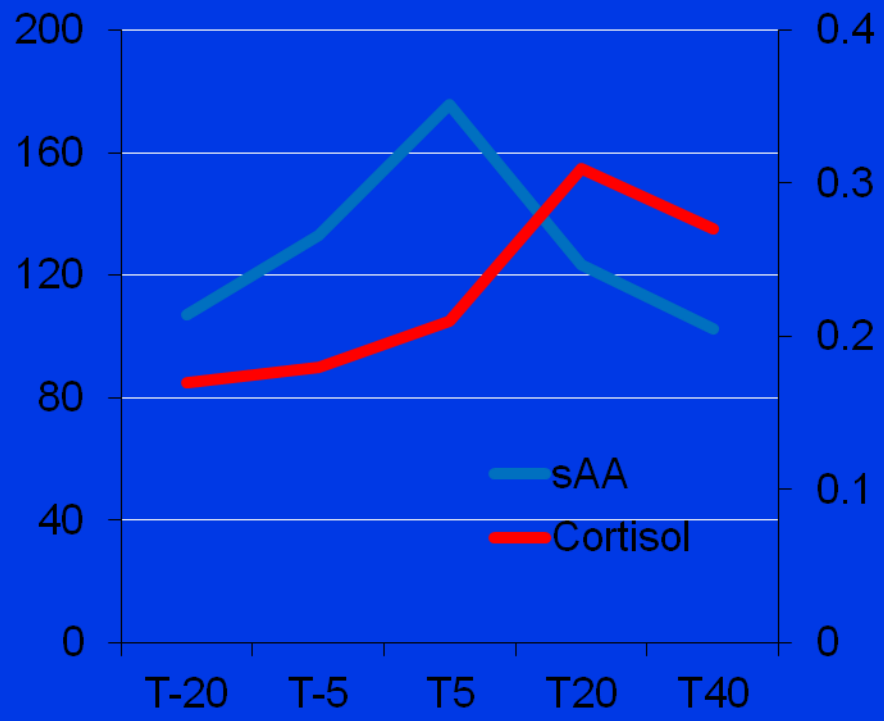
Full Term (≥37 weeks gestation) Premature (<37 weeks gestation)

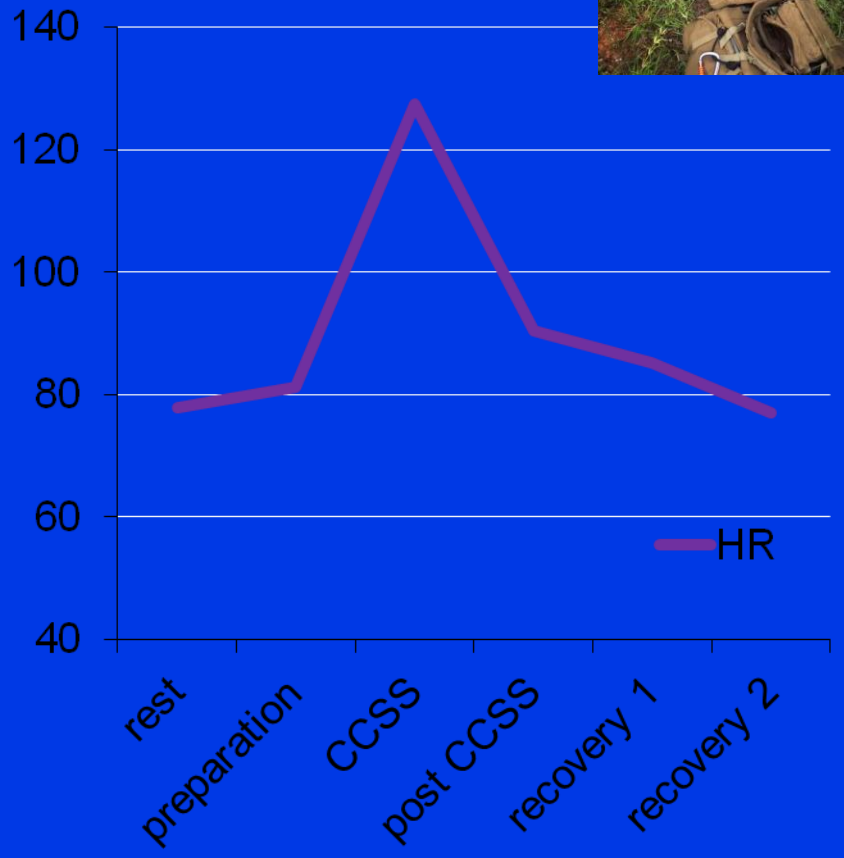
Sullivan, Mills, Winchester, Granger (in prep)

HPA Stress Reactivity In Adulthood by Birth Group



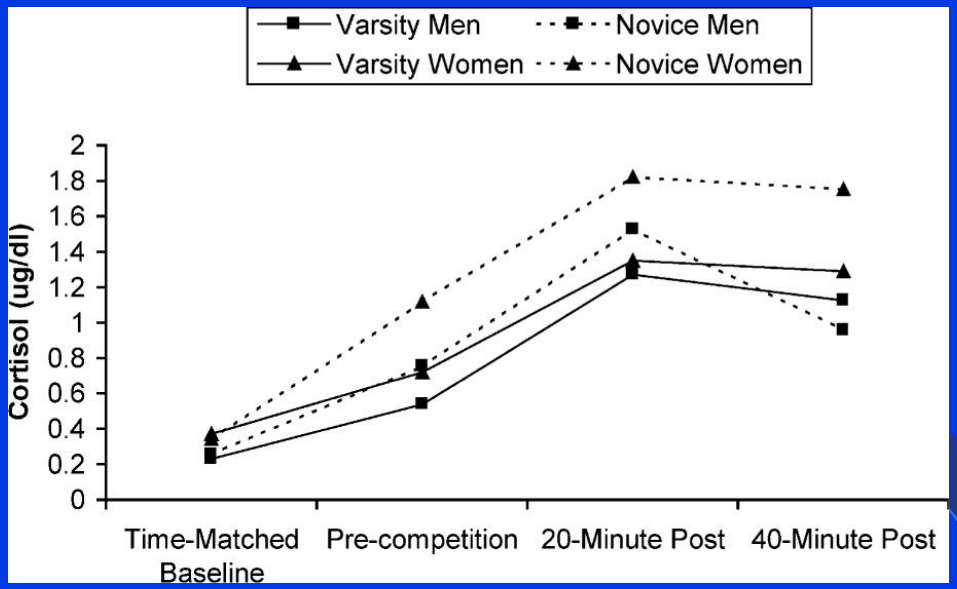
Army Nurse Combat Casualty Stress Task



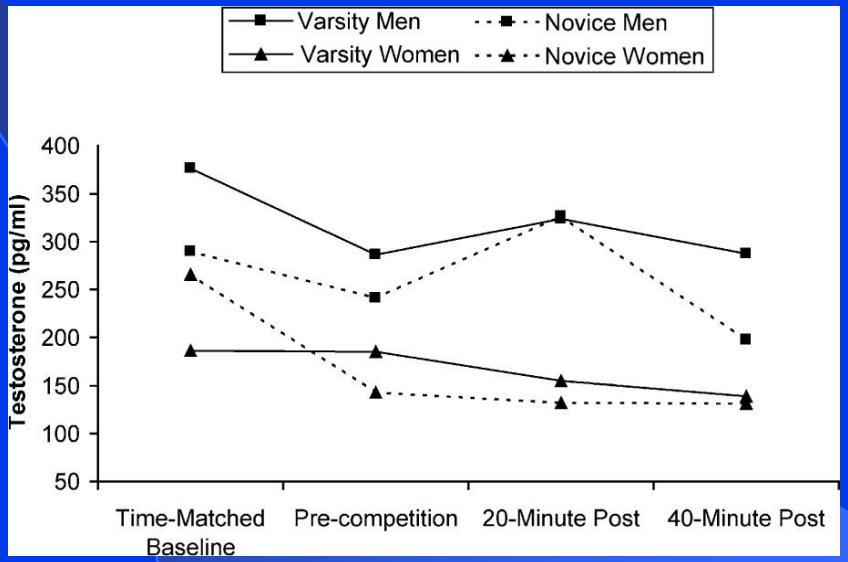


McGraw et al. (2012) Psychoneuroendocrinology

Salivary Cortisol Response to Rowing Ergometer Competition



Men and women's endocrine responses were more different than alike and varied by level of experience



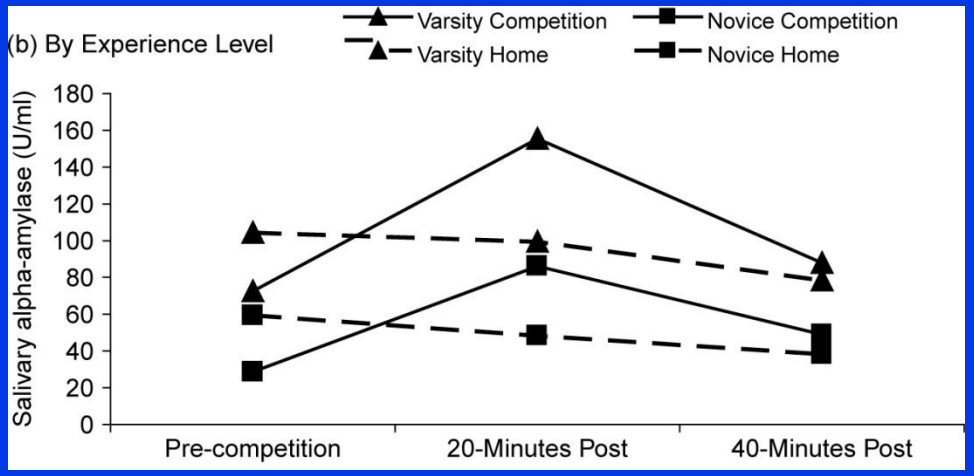
Individual differences associated with social Afiliation rather than dominance or competitiveness

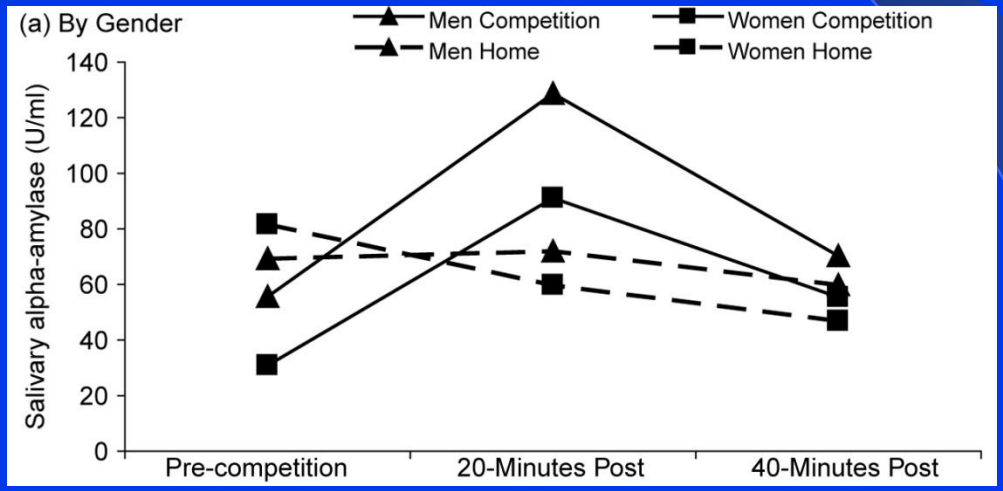
Kivlighan, Granger, Booth (2005). *Psychoneuroendocrinology*

Salivary sAA Response to Rowing Ergometer Competition

sAA higher for varsity than novice, and associated with performance.

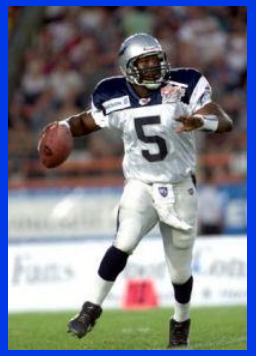
sAa reactivity associated with perceived dominance



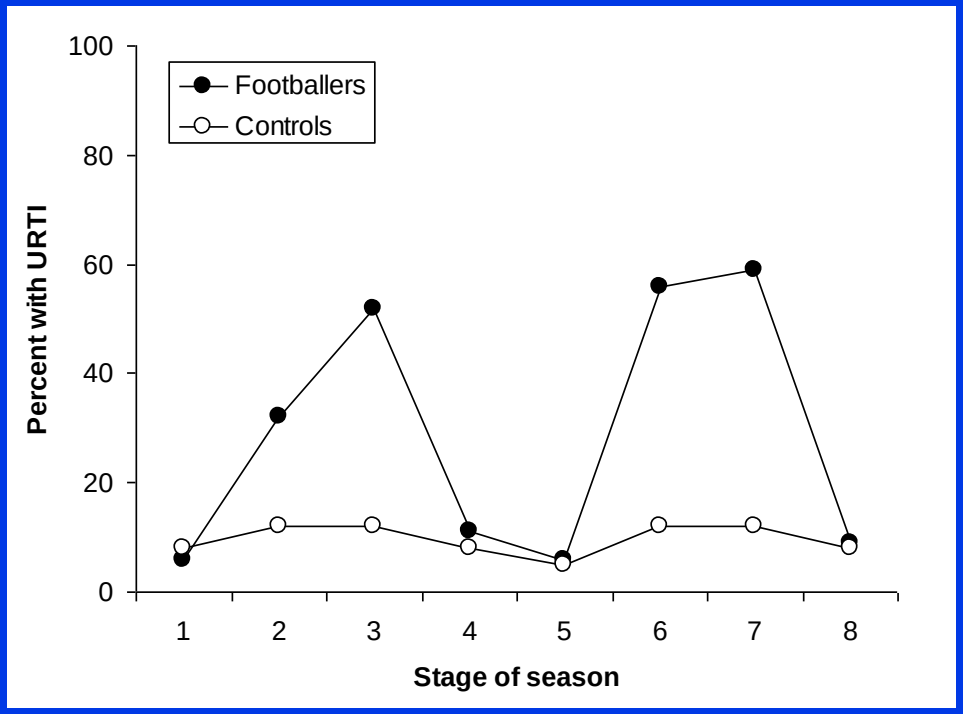


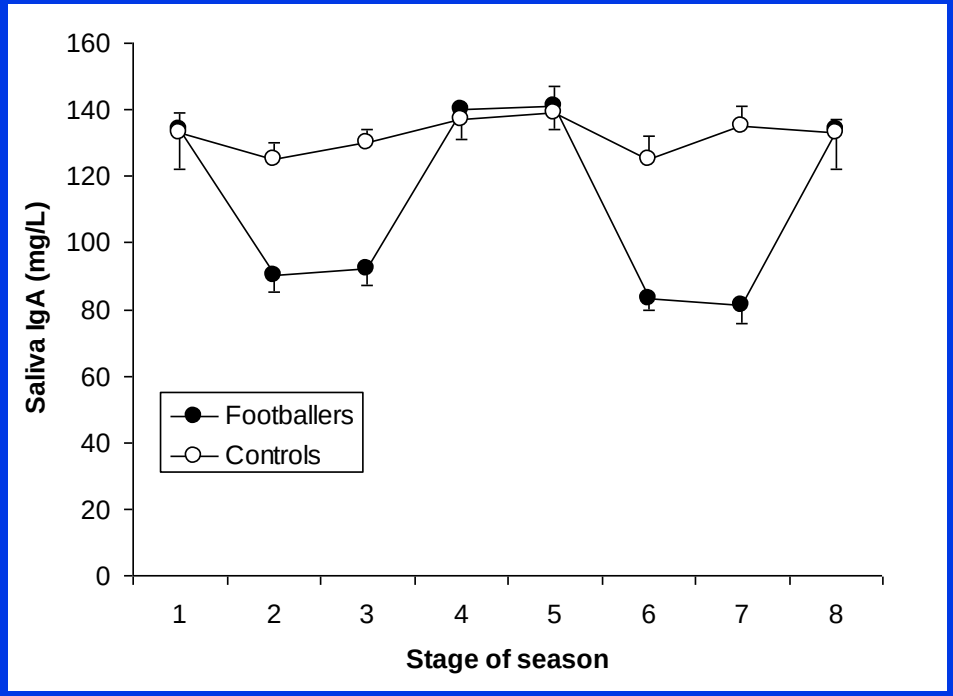
sAA reactivity specific to competition, and levels Higher in men than women

Kivlighan & Granger (2006) Psychoneuroendocrinology



Mucosal IgA and URTI in American College Football Players: A Year Longitudinal Study



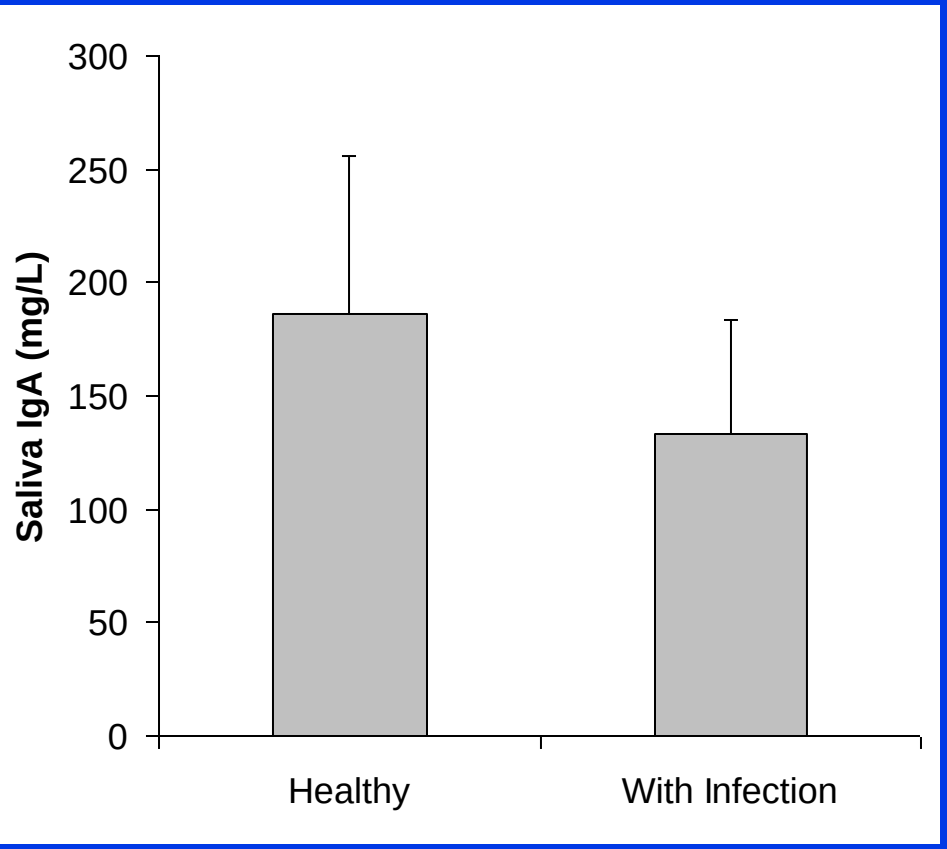


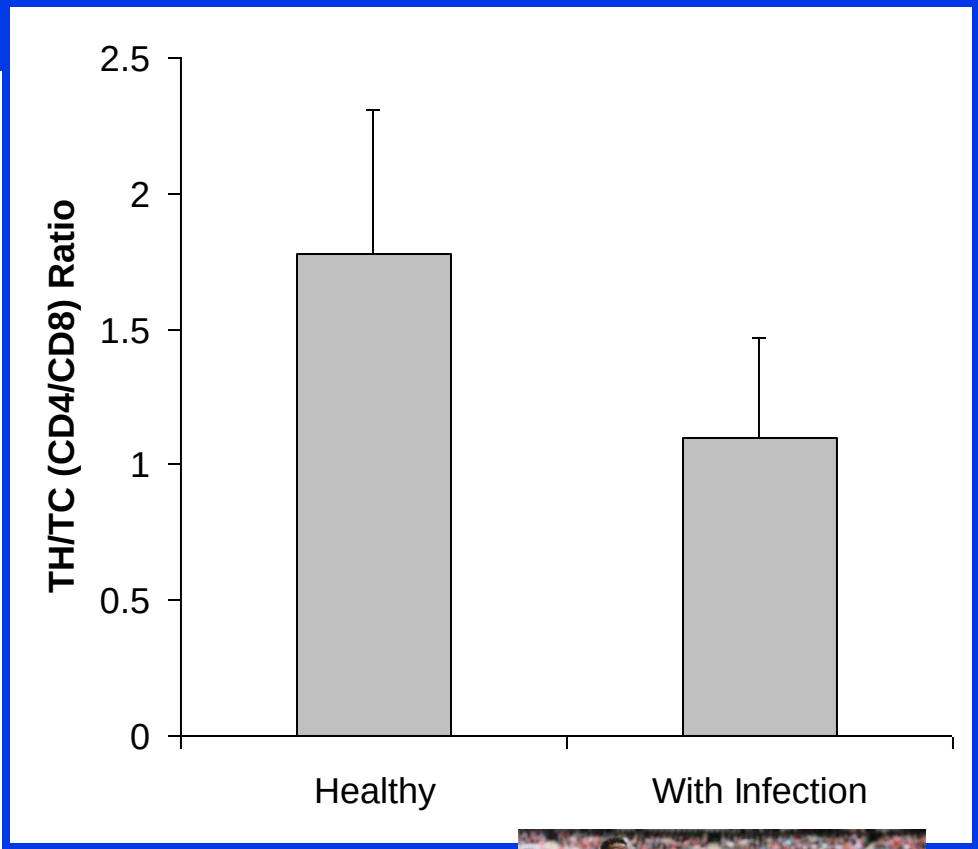
Autumn Winter Spring

Fahlman & Engels (2005) Med Sci Sports Exerc 37(3): 374-380

Immunological differences in English Premier League football players with infections

28 players in total were sampled on up to 4 occasions (total of n=94); 5 players had viral infections on 11 occasions





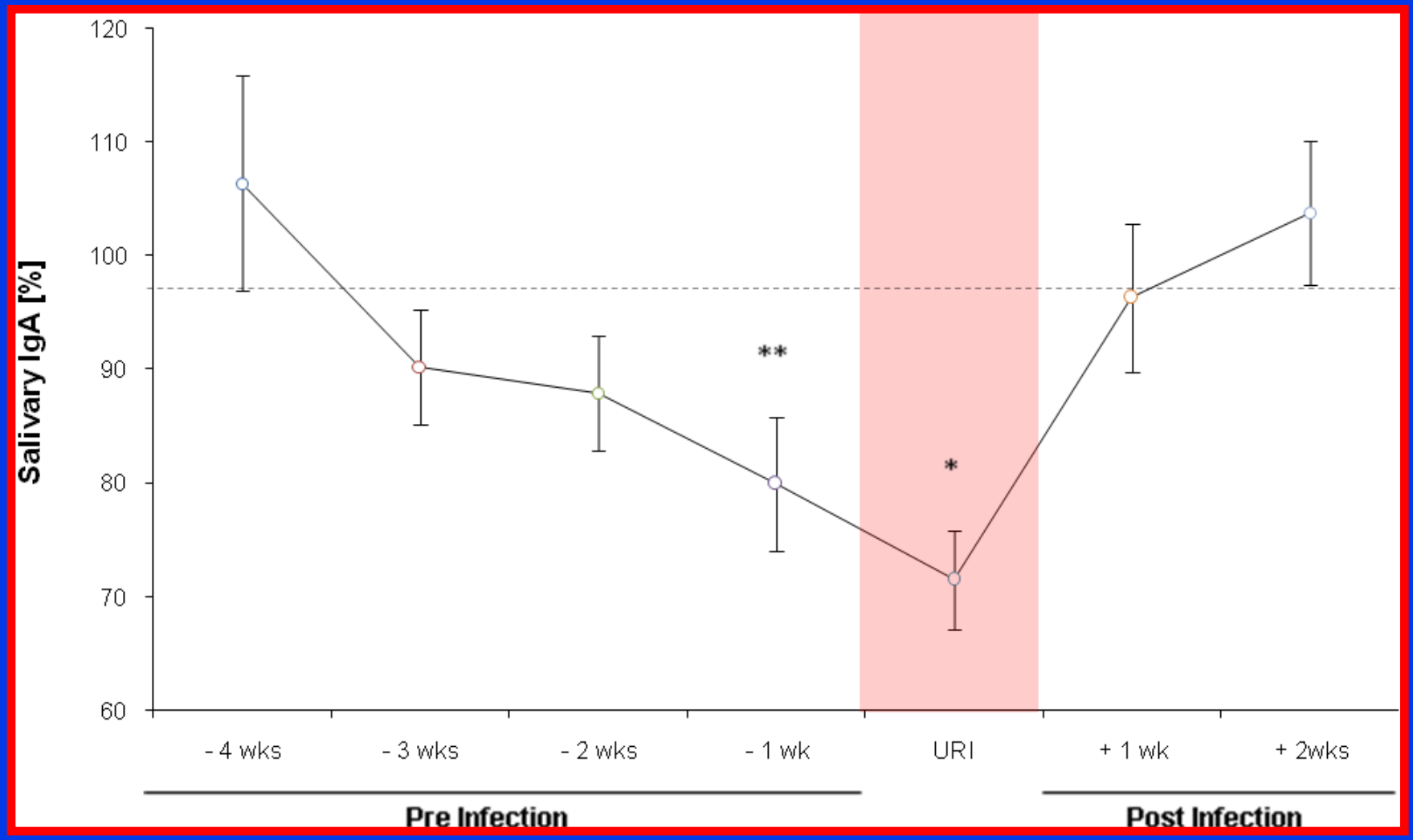
Gleeson

A longitudinal study of changes in s-IgA and respiratory illness in athletes

- 38 members of America's Cup yacht crew
- Studied over 50 weeks of training and sailing
- Morning saliva samples collected weekly
- Clinically confirmed illness

Neville, Gleeson & Folland (2008) *Med Sci Sports Exerc 40(7):1228-1236*

Relative s-IgA before and after an infection episode



Gleeson

Conclusions

- On a group basis, relative s-IgA determined a substantial proportion of the variability in weekly infection incidence
- Significant reduction in s-IgA in the 3 weeks prior to infection
- Relative s-IgA value <40% of healthy baseline value indicated a 50% chance of contracting an infection within 3 weeks

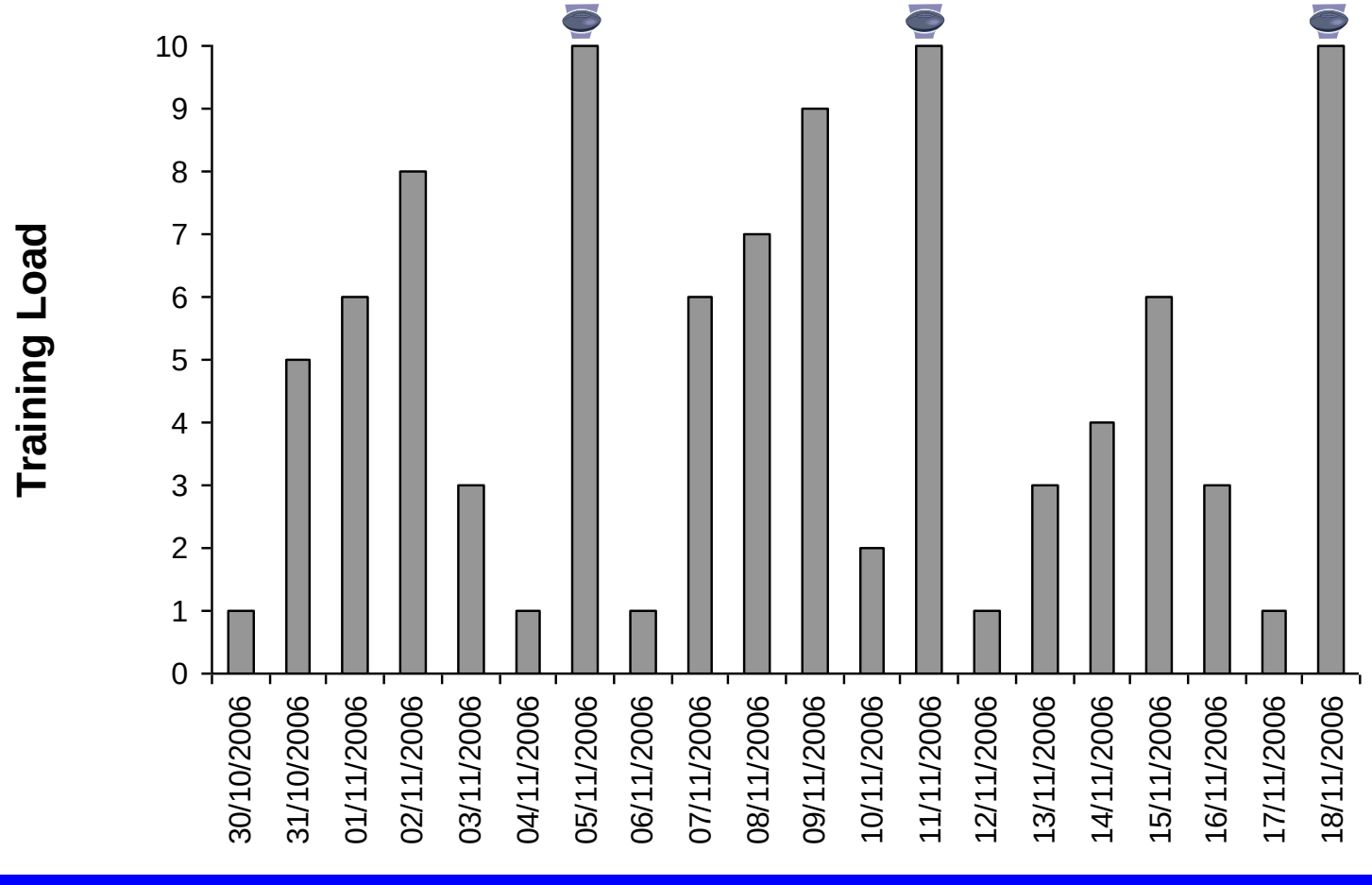
Hence, possible predictive value with regular monitoring

Monitoring hormones

- With stress: ↑ C and ↓ T
- C/T ratio ↑ 30% = Overreached/Stressed
- Salivary C/T (1000*nM/pM) ratio > 40
  = Stressed or Not Recovered

Monitoring recovery in RFU players

- 10 England International RFU Players
- 29 1 years, 102.4 3.9 kg, 1.86 0.02 m
- 4 saliva samples collected each wk for 3 weeks
- Morning collection, before breakfast
- IgA, Cortisol, Testosterone measured by ELISA
- Transferrin (blood contamination) by ELISA
- Training load monitored
- 3 international matches
- Players' perceptions of fatigue and stress monitored using the REST-Q questionnaire


Training Load
10
9
8
7
6
5
4
3
2
1
0
30/10/2006
31/10/2006
01/11/2006
02/11/2006
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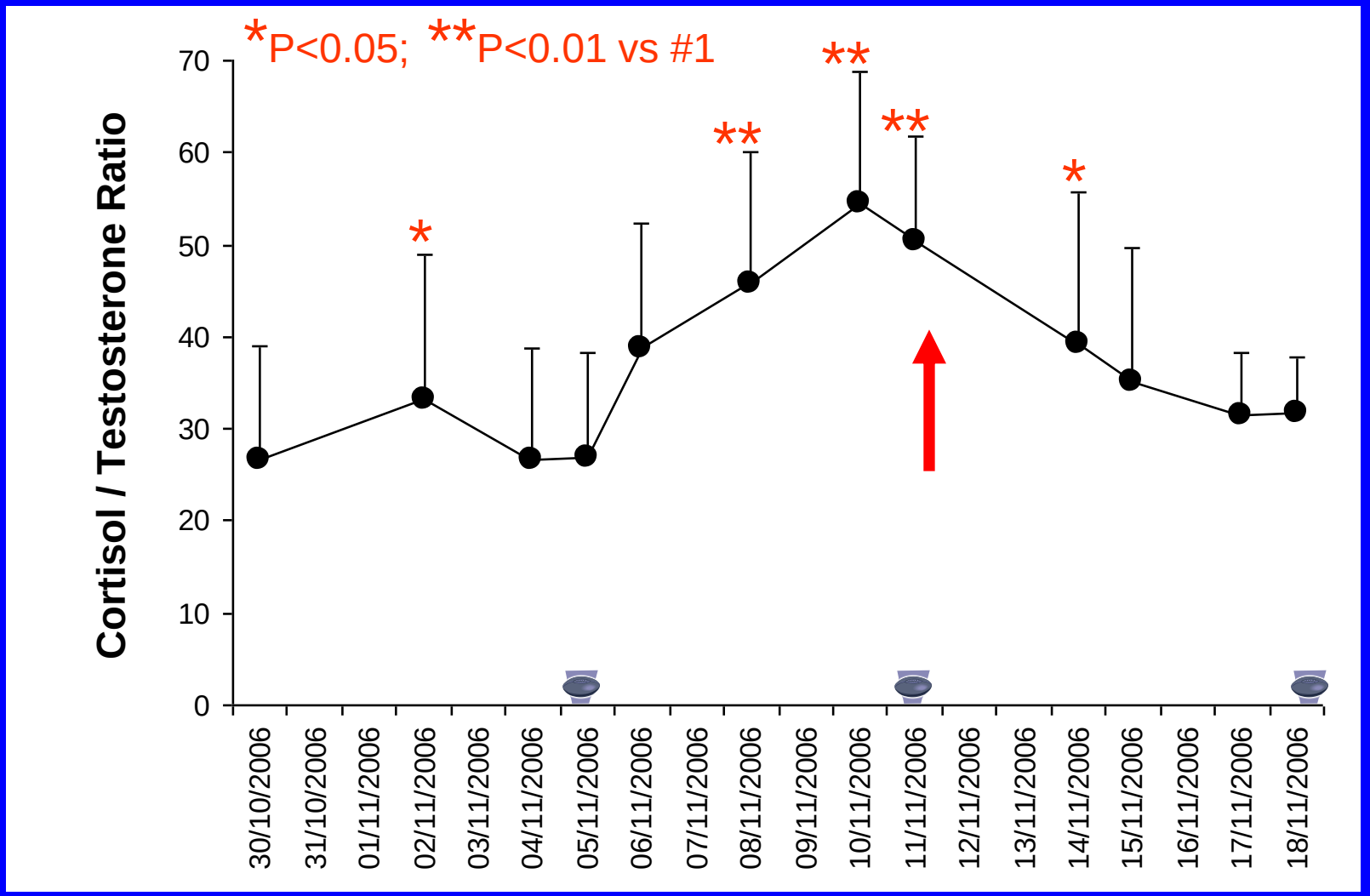


Effect of time (P<0.001)

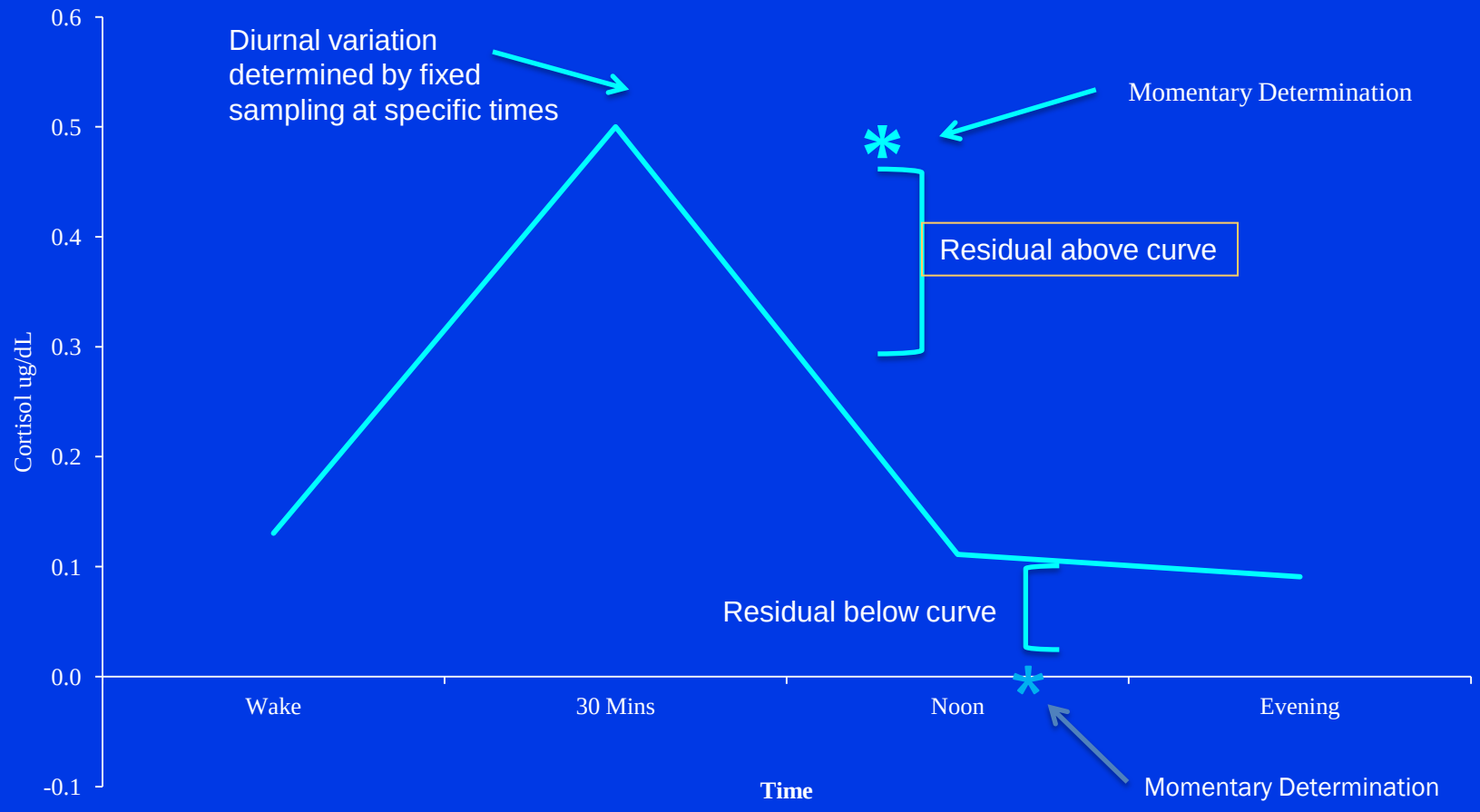
Conclusions

- On a group basis, saliva C/T ratio appears to be indicative of stress or inadequate recovery
- May help in recovery prescription for individual players
- S-C/T >40 likely associated with underperformance

Hence, possible value with monitoring during intensive phases of season

Gleeson

Momentary Cortisol - Behavior Associations in Everyday Contexts



Dyadic Similarity in Cortisol Levels

	Mother	Father	Middle Child
Mother			
Father	.34** (326)		
Middle Child	.19** (320)	.21** (326)	
Adolescent	.34** (320)	.24** (322)	.22** (318)

** P<.01

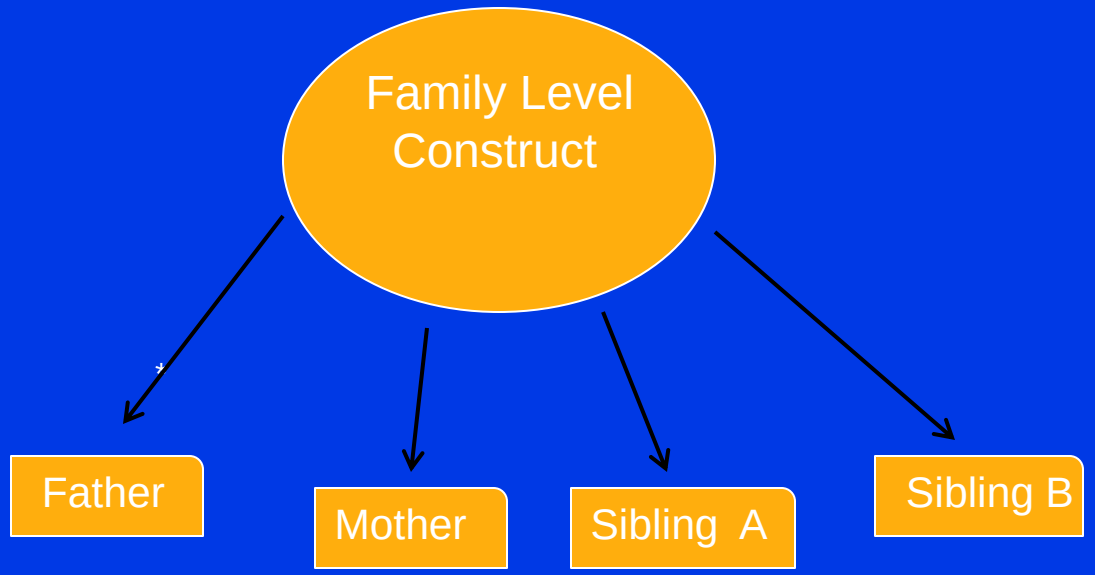
400 Mothers, Fathers, Older and younger sibling
Saliva sampled at home two mornings
Relationship Quality Moderates
Martial satisfaction and conflict, spouse who are congruent have higher covariation in cortisol levels

Booth, Johnson, Goslin, & Granger (in prep)

Commonality among members of social groups

Latent Level

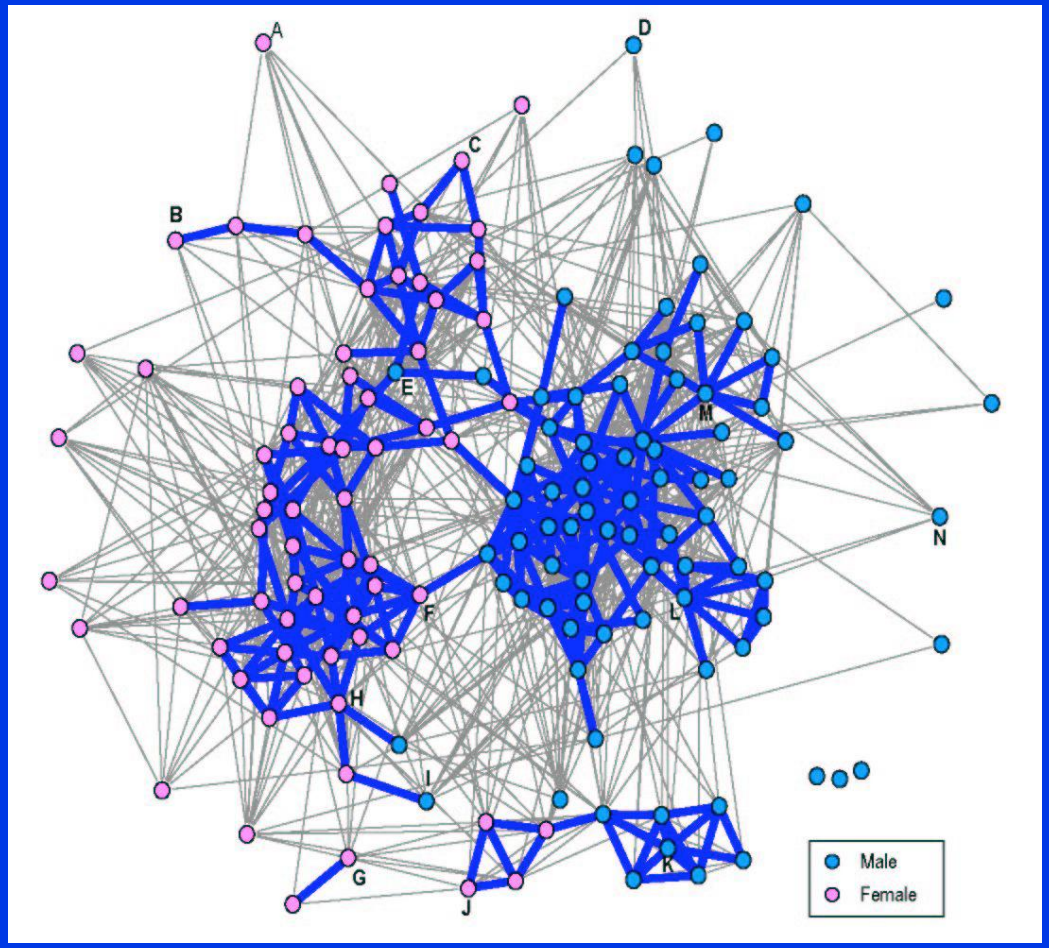
Manifest Level



Approach would theoretically enable the exploration of factors that contribute to each individual members contribution to the family level construct, and contribute to differences between groups.

Social Networks: Families, Teams, Classes

Self-reported Friendship Nominations in 6th Grade



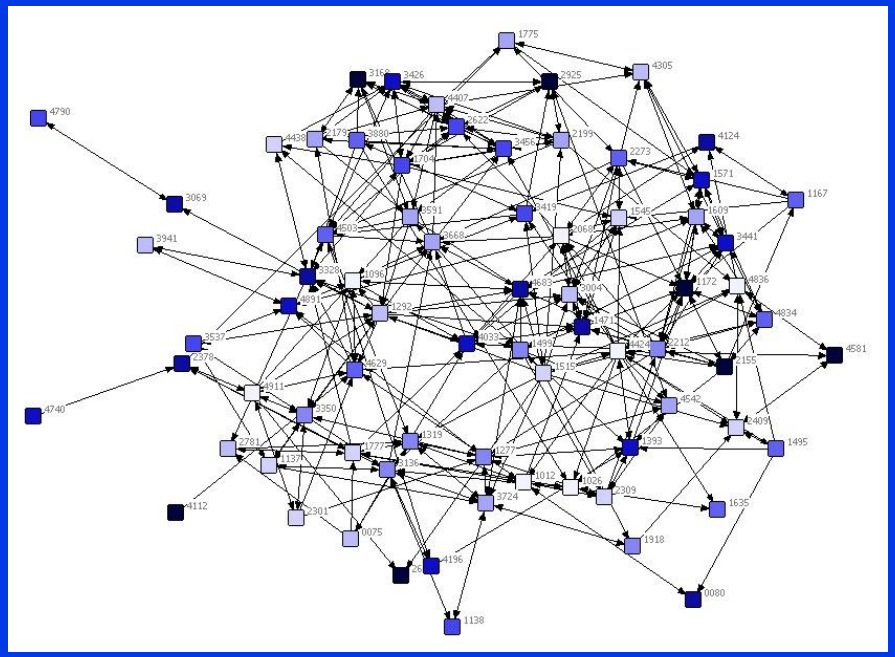
Thick blue lines are reciprocated Friendships

Thin grey lines are asymmetric Friendships

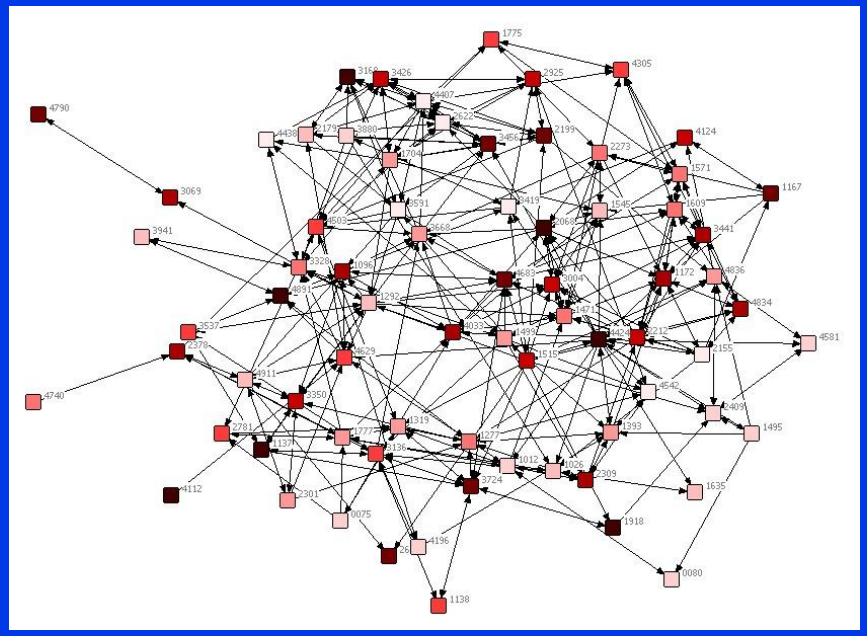
Data provided by Scott Gest, PhD, Penn State University

Cortisol, Testosterone, and the Social Network: Friendship Nominations

Cortisol



Testosterone



Assessing genetic polymorphisms using DNA extracted from cells present in saliva samples.

Are the typical volumes of saliva collected in studies of child development sufficient to enable isolation of DNA for genetics analyses?

Do collection device materials affect the isolation of sufficient quantity and quality of DNA from saliva?

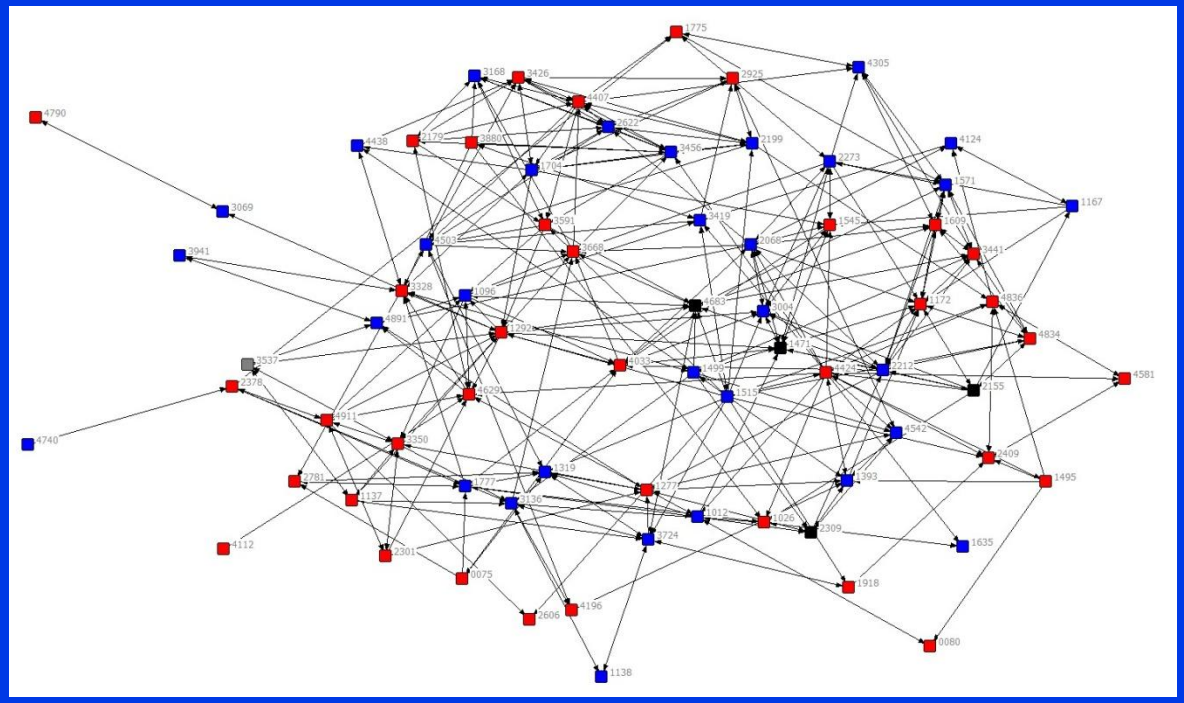
What are the effects of room temperature (RT) storage of saliva samples on the quality and quantity of DNA recovered?

Do archived saliva samples that have been exposed to multiple freeze-thaw cycles yield sufficient quantity and quality of DNA for genetic analyses?

When oral fluids are collected from specific areas in the mouth associated with different salivary glands, is there a difference in DNA quantity and quality?

Nemoda, et al. (2012). *BMC Medical Research Methodology*.

OXTR and the Social Network: Friendship Nominations



AA – Black; AG – Red; GG - Blue

Epigenetics

- Global Methylation -- Levels correlated in DNA extracted from cells from oral fluid compared to DNA extracted from cells from whole blood

Cytokines

- Design: 113 Healthy Adolescent Girls, multiplex EC Immunoassay, 3 waves of annual samplings
- GM-CSF, IFNγ, IL-1ß, IL-2, IL-6, IL-8, IL-10, IL12-p70, and TNFa.
- Cytokine levels: all cytokines, except IFNγ and IL-10, were detectable in the majority of saliva samples; non-normal distributions; levels of all cytokines lower in saliva than serum except IL-8 and IL-1ß; saliva levels of IL-8 and IL-1ß were >20x higher than other salivary cytokines;
- Cytokine Inter-correlations : in saliva were lower than in serum; individual differences in cytokine levels were more stable across years when measured in serum than in saliva;
- Stability : there were strong associations between salivary adiponectin and cotinine with salivary but not serum cytokines; and
- Serum-saliva correlation: only IL-1ß
- Correlates: Salivary cytokine levels were not associated with age, pubertal development, or gynecological age,
- Conclusion: Variation in salivary cytokine levels largely reflects compartmentalized local activity of the oral mucosal immune system raising questions about the role they play, if any, in intracellular and hormonal signaling within the neuro-endocrine-immune network.

Granger et al (in prep)

Prospective Markers of TBI in Saliva

Name	Type	Primary Source	Primary Function/Use	Size
GFAP	filament and cytoskeleton protein	astrocytes	marker of astrocytes, astrocyte differentiation, astrogliosis, tumor marker	45 kDa
S100B	calcium-binding protein	cytoplasm of astroglia and Schwann cells	participates in intracellular activities, marker of pathological conditions and injury	20 kDa
NSE	glycolytic enzyme	cytoplasm of neurons, neuroendocrine cells	released under pathological conditions, marker of cell damage, tumor marker	78 kDa
Neurogranin	neuroprotein, protein kinase C substrate	neurons	participates in synaptic signaling, marker of dendrite spine density and synaptic plasticity	7.5 kDa
BDNF	neurotrophin	widespread in CNS	marker related to neuron growth and survival	14 kDa
Beta-Synuclein	soluble protein	presynaptic brain tissues, astrocytes	development and maintenance of synaptic function, tumor marker	19 kDa
ICAM-5	transmembrane glycoprotein	neurons	synapse development, immune and inflammatory responses	130 kDa
MT-III	metal-binding protein	astrocytes	protects neurons and other tissues against toxic factors, oxidative stress	6 kDa


(c) sharon loadbitter


PNC

Future Directions

- Salivary bioscience is poised to enable and facilitate advances in multiple fields
- Emerging applications have implications for screening and diagnostics, and decisions related to personal health, well being, and performance
- Prevention and intervention science
- Community of interdisciplinary scholars linked together by an international network of centers of excellence

Questions and Discussion

Johns Hopkins University

Center for Interdisciplinary Salivary Bioscience Research

SPIT camp
Johns Hopkins