Aminoglycoside-derived Liposomes for Synergistic Drug Delivery

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Cancer Diseases

Estimated New Cases

Males				Females		
Prostate	220,800	26%		Breast	231,840	29%
Lung & bronchus	115,610	14%		Lung & bronchus	105,590	13%
Colon & rectum	69,090	8%		Colon & rectum	63,610	8%
Urinary bladder	56,320	7%		Uterine corpus	54,870	7%
Melanoma of the skin	42,670	5%		Thyroid	47,230	6%
Non-Hodgkin lymphoma	39,850	5%		Non-Hodgkin lymphoma	32,000	4%
Kidney & renal pelvis	38,270	5%		Melanoma of the skin	31,200	4%
Oral cavity & pharynx	32,670	4%		Pancreas	24,120	3%
Leukemia	30,900	4%		Leukemia	23,370	3%
Liver & intrahepatic bile duct	25,510	3%		Kidney & renal pelvis	23,290	3%
All Sites	**848,200**	**100%**		**All Sites**	**810,170**	**100%**

Estimated Deaths

Males				Females		
Lung & bronchus	86,380	28%		Lung & bronchus	71,660	26%
Prostate	27,540	9%		Breast	40,290	15%
Colon & rectum	26,100	8%		Colon & rectum	23,600	9%
Pancreas	20,710	7%		Pancreas	19,850	7%
Liver & intrahepatic bile duct	17,030	5%		Ovary	14,180	5%
Leukemia	14,210	5%		Leukemia	10,240	4%
Esophagus	12,600	4%		Uterine corpus	10,170	4%
Urinary bladder	11,510	4%		Non-Hodgkin lymphoma	8,310	3%
Non-Hodgkin lymphoma	11,480	4%		Liver & intrahepatic bile duct	7,520	3%
Kidney & renal pelvis	9,070	3%		Brain & other nervous system	6,380	2%
All Sites	**312,150**	**100%**		**All Sites**	**277,280**	**100%**

Cancer statistics, 2015.
Siegel RL, Miller KD, **Jemal** A.
CA **Cancer** J Clin. **2015**;65(1):5-29.

> 2 women die of breast cancer every day in AZ

Triple Negative Breast Cancer (TNBC)

- **"Triple-negative": Lack of estrogen, progesterone, and HER2 receptors → no targeted therapies.**

  Toh TB et al. *Mol Pharm*. 2014, 11(8), 2683-91

- **Diagnosed in 15-30% of all breast cancer cases. Aggressive and high mortality**

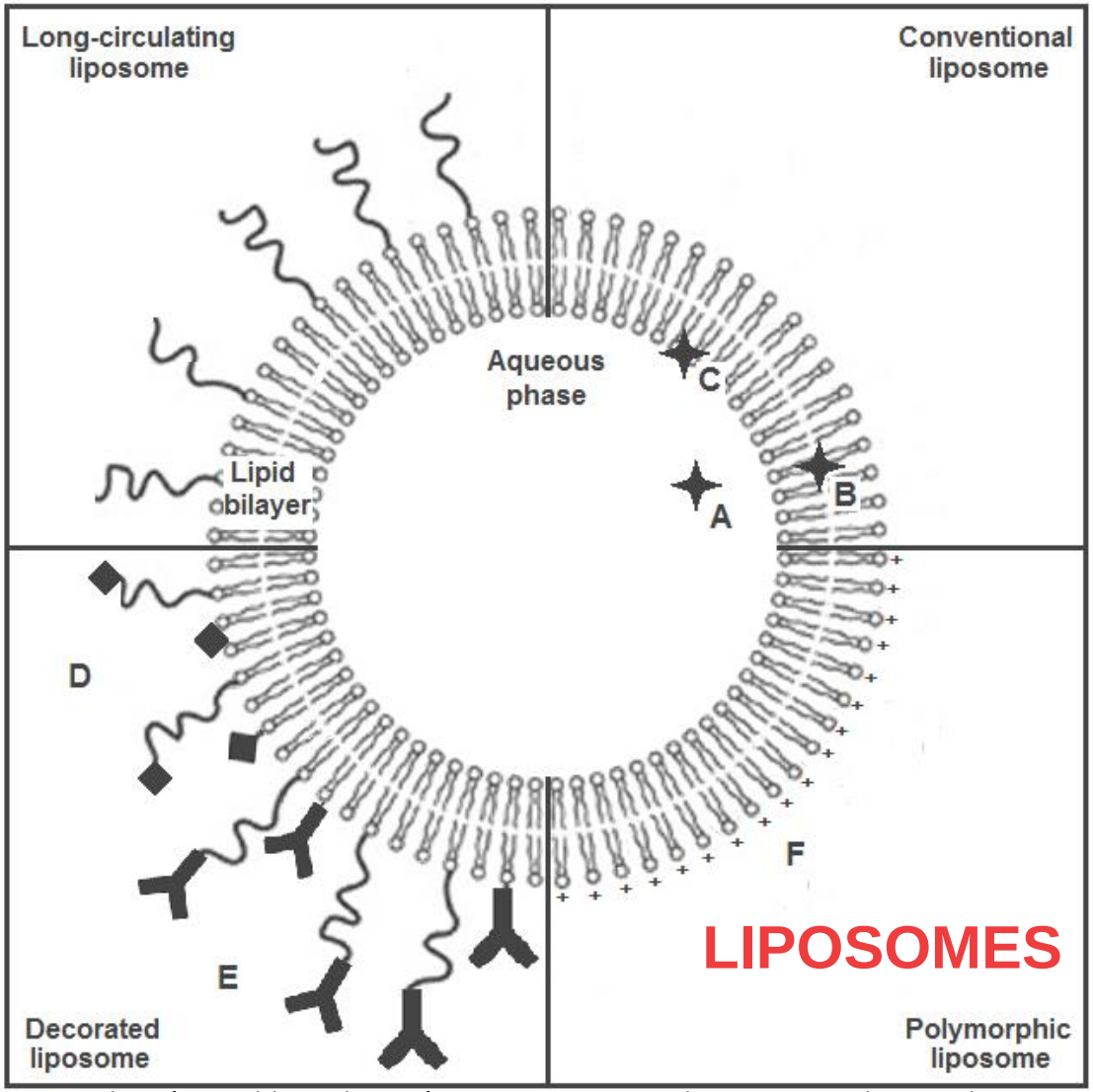
- **Standard treatment: surgery with adjuvant chemotherapy and radiation therapy.**

  Crown et al. *Annals of Oncology*, 2012, 23, vi56–vi65.

No targeted strategies for TNBC in the clinic

Urgent need for effective and targeted therapeutics for TNBC

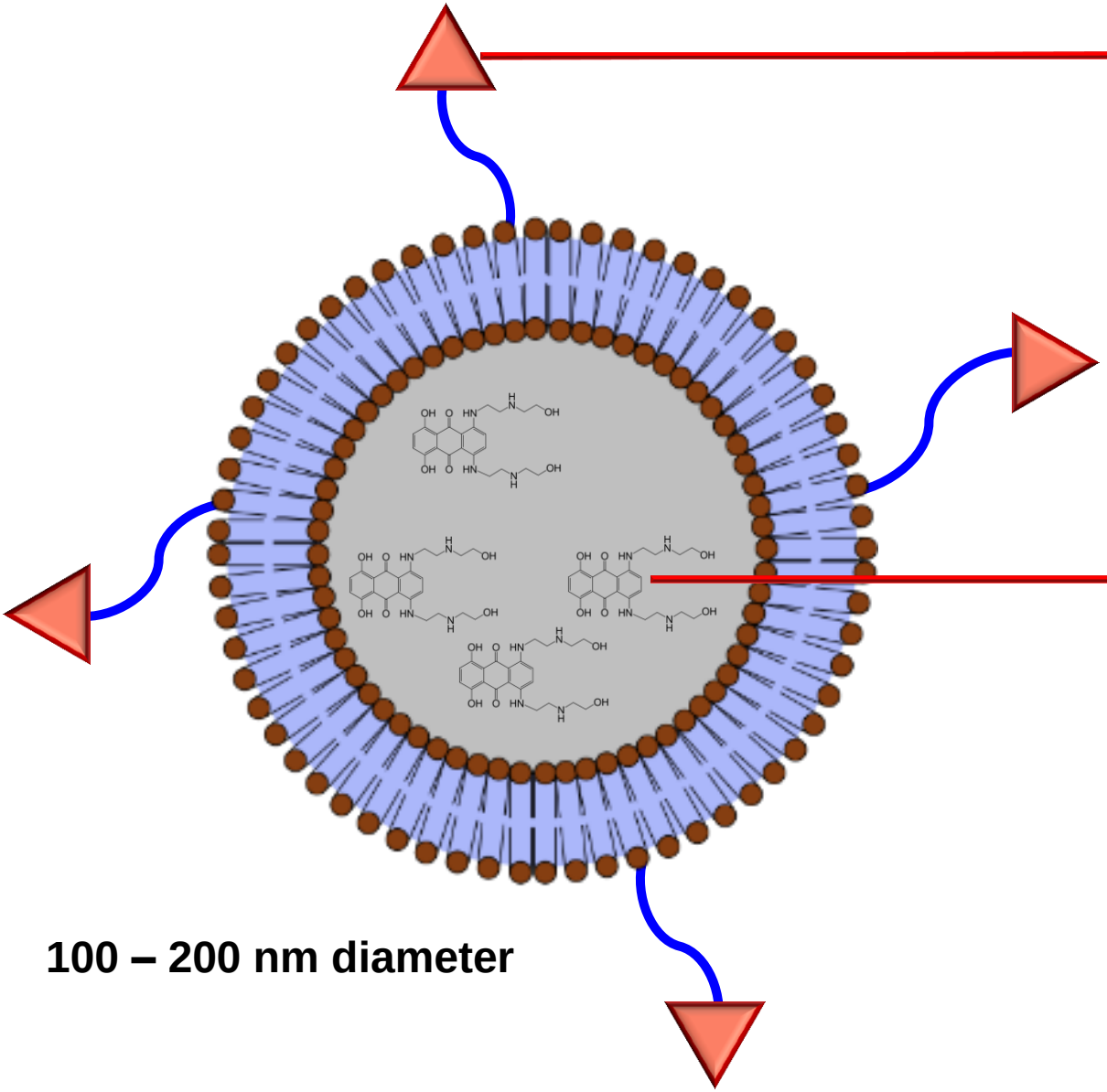
Nanoparticle-mediated Drug Delivery



1. Can **minimize cardiotoxicity** (e.g. FDA-approved Doxil®)
2. Can Facilitate
   i. Longer **circulation time** in the body
   ii. **Targeted delivery** to tumors
   iii. Delivery of **multiple drugs**

Figure credit: Sávia Caldeira de Araújo Lopes, Cristiane dos Santos Giuberti, Talita Guieiro Ribeiro Rocha, Diêgo dos Santos Ferreira, Elaine Amaral Leite and Mônica Cristina Oliveira (2013). Liposomes as Carriers of Anticancer Drugs, Cancer Treatment - Conventional and Innovative Approaches, Prof. Letícia Rangel (Ed.), ISBN: 978-953-51-1098-9, InTech, DOI: 10.5772/55290. Available from: http://www.intechopen.com/books/cancer-treatment-conventional-and-innovative-approaches/liposomes-as-carriers-of-anticancer-drugs

Our Proposed Approach



100 – 200 nm diameter

Agent for targeting TNBC cells

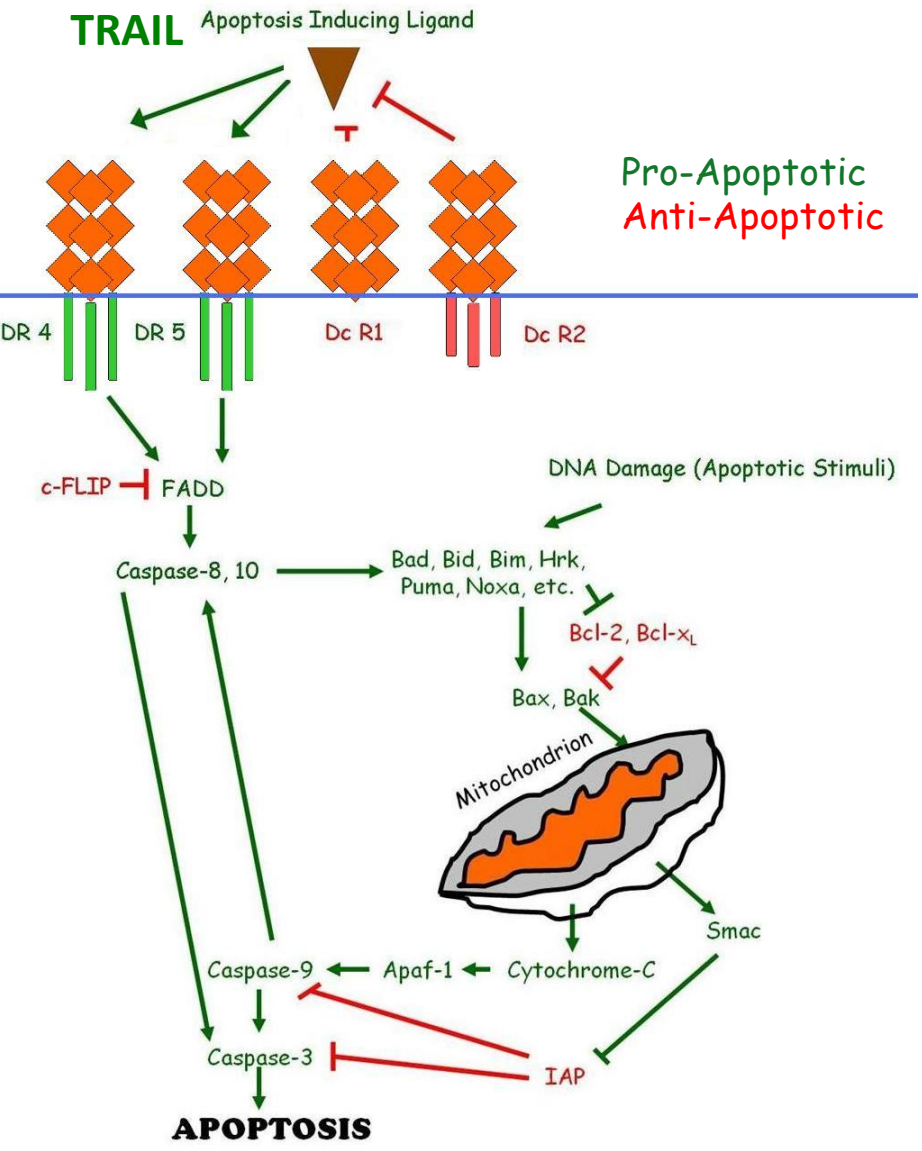
e.g. folic acid and antifolates

Encapsulated single or synergistic drug combinations

e.g. Mitoxantrone(*) (FDA-approved)

(*) Evison BJ et al. Med Res Rev. **2016**, 36(2), 248-99

Background Studies in the Rege Lab - I



TRAIL selectively kills cancer cells but

Cells are / can develop a resistance to TRAIL

We identified the combination of mitoxantrone + TRAIL as a novel synergistic treatment in multiple cancer cell lines(*)

Micelles (~15 nm; untargeted) were used to deliver mitoxantrone to cancer cells(#)

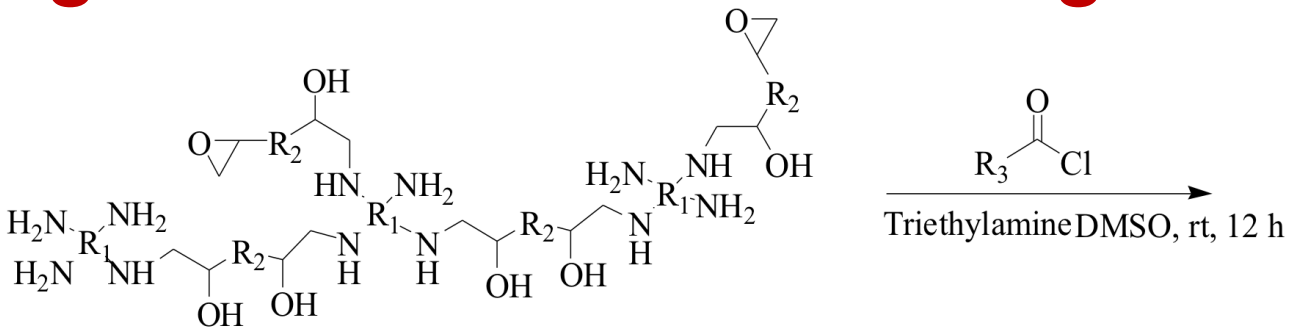
Poor stability

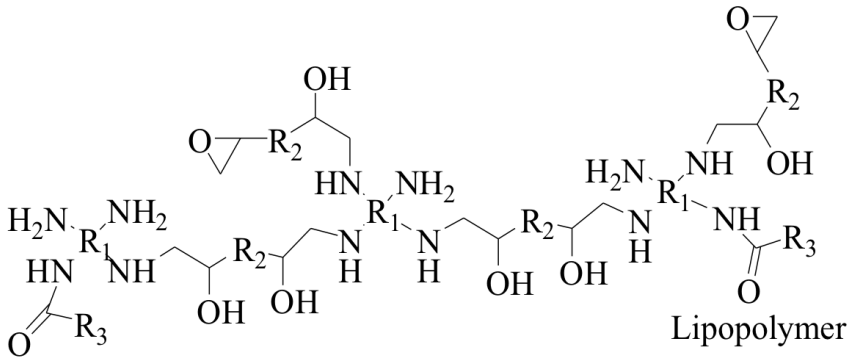
Difficult to load multiple drugs

(*) *BMC Cancer*. 2011; 11: 470.

(#) *Nanomedicine-UK*. 2014; Vol. 9: 1775-1788.

Background Studies in the Rege Lab - II





R_1= Aminoglycoside Monomer
R_2= Glycerol diglycidyl linker
R_3= Alkyl group(C_6,C_{14},C_{18})

Aminoglycosides are amine-containing sugars used as antibiotics (e.g. neomycin in Neosporin®)

Mol. Pharm. **2009; 6: 86-97.**

Biomaterials ***2014; 35: 1977 - 1988.***

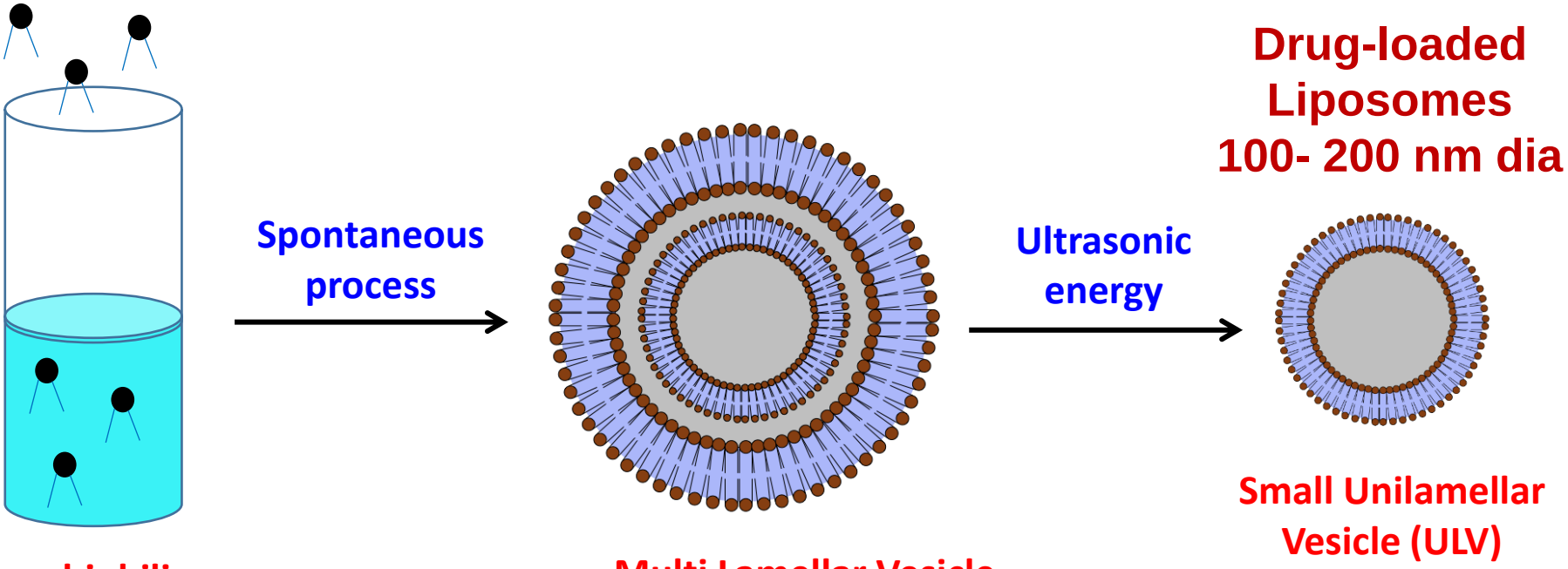
J. Control. Release ***2014; 176: 35–43.***

Int. J. Pharm*. 2015; 489: 18-29.*

ACS Biomater. Sci. Eng*., 2015; 1: 656–668.*

Aminoglycoside Lipopolymers ➔ Mitoxantrone-loaded Liposomes

- **Aminoglycoside Lipopolymers + Co-lipids and Drug (Mitoxantrone) dissolved in chloroform/methanol and dried.**
- **Swollen overnight in water.**
- **Vortexed and sonicated for 1-2 minutes**



Liposomes

- **Five different types of mitoxantrone-loaded aminoglycoside liposomes**
- Hydrodynamic Diameters: **110 – 160 nm**
- Zeta Potential Values: **+ 32-36 mV (positively charged)**

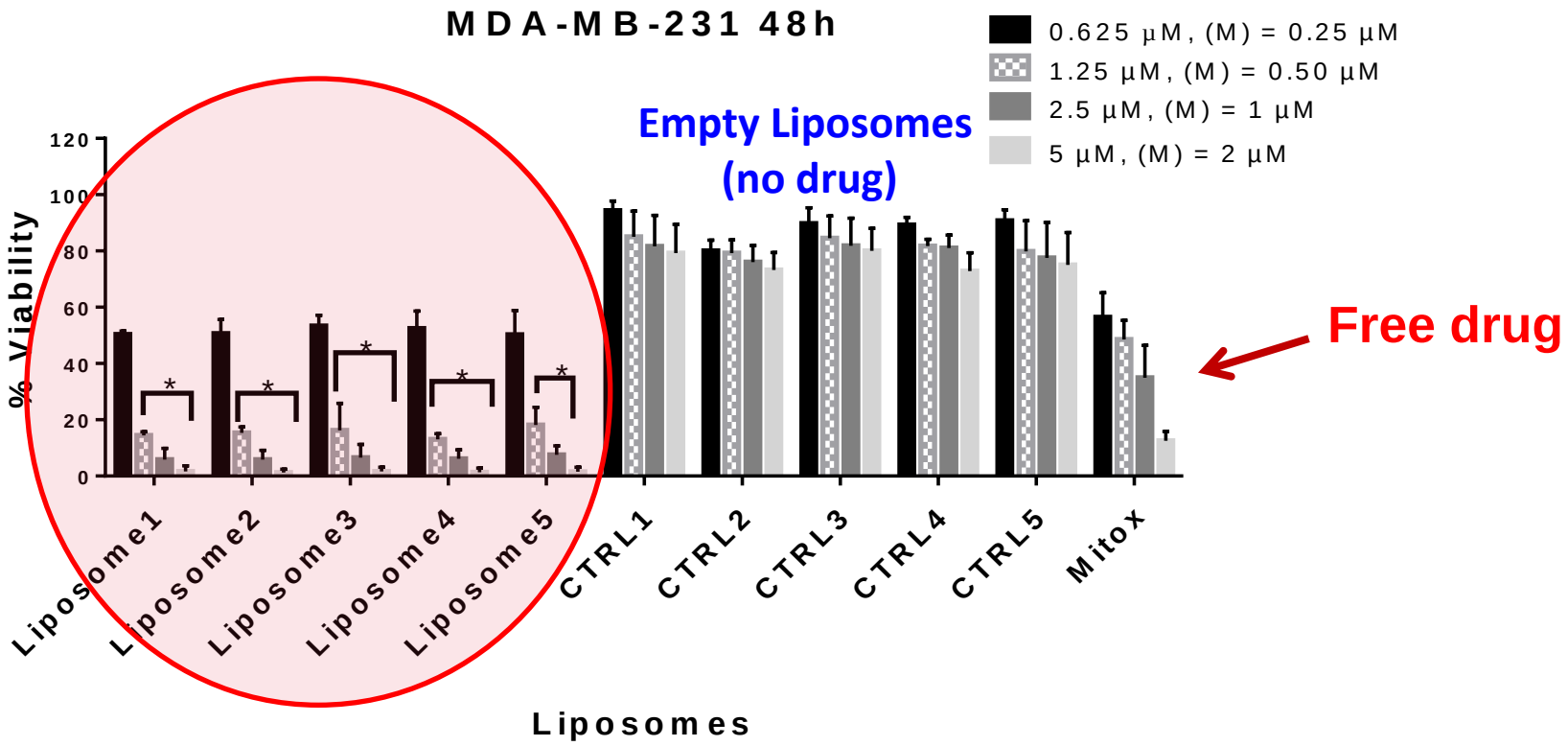
Liposome Formulation	HYDRODYNAMIC DIAMETER (nm)		Zeta potential (mV)	
	One month	Two months	One month	Two months
1	132 ± 1	128 ± 1.9	31 ± 3	30 ± 1.3
2	127 ± 1.5	132 ± 1.3	28 ± 3.3	27 ± 2.6
3	138 ± 1.9	136 ± 1.5	32 ± 4.3	30 ± 1.8
4	158 ± 2	140 ± 1.9	31 ± 6.8	32 ± 3.3
5	143 ± 1.3	135 ± 1.7	29 ± 6.7	28 ± 4.9

Stable Drug-loaded Nanoparticles

I.

Mitoxantrone-loaded liposomes (Single-agent)

TNBC Cells: Mitoxantrone Liposomes

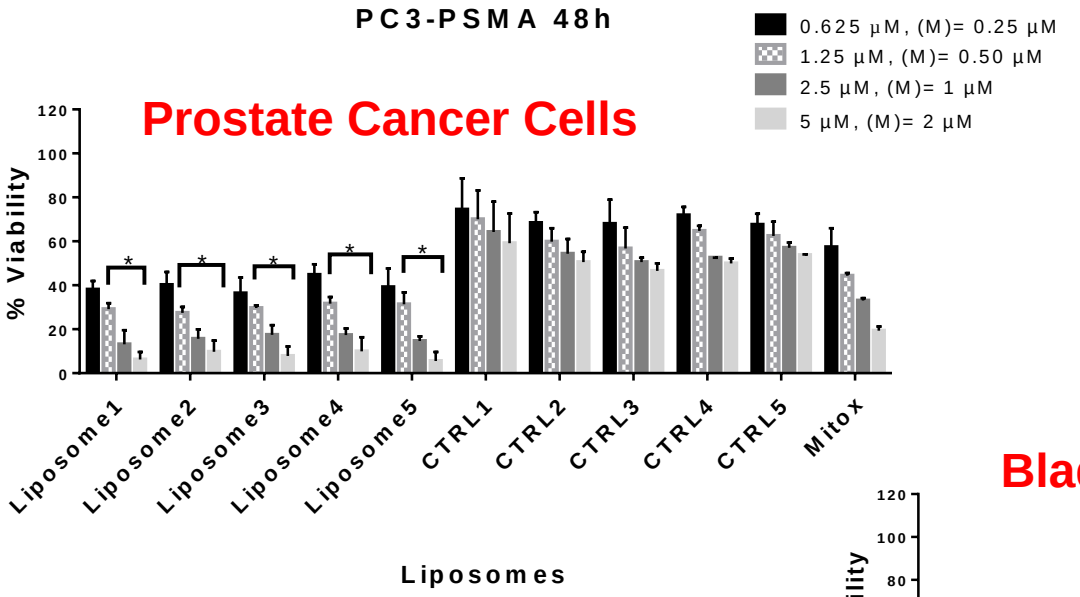


CTRL: liposomes without drug (empty liposomes)

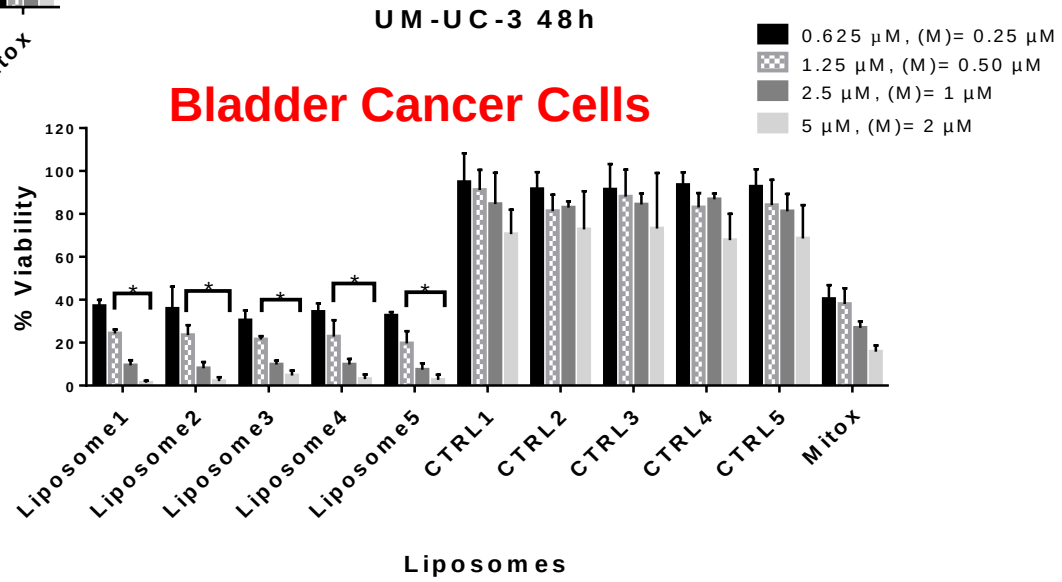
Mitox: free (unencaspulated) mitoxantrone

Near-complete ablation of TNBC cells in culture

Prostate and Bladder Cancer Cells



* $p \leq 0.05$, Compared Mitoxantrone containing liposomes Vs Free Mitox



Mitoxantone-Liposomes are also Effective in Prostate and Bladder Cancer Cells

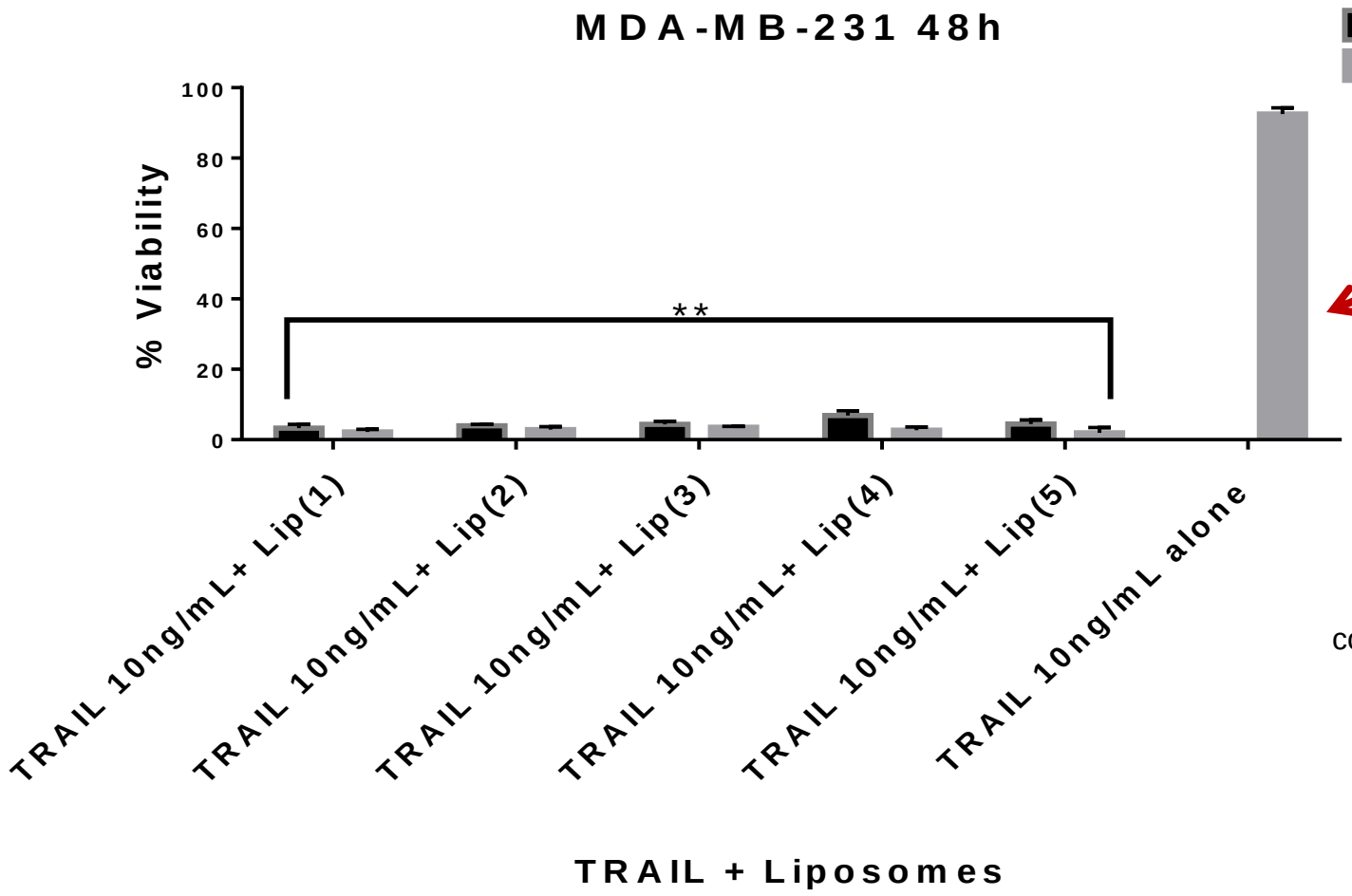
II.

Efficacy of Mitoxantrone-loaded liposomes

+

TRAIL protein (delivered separately)

TNBC Cells: Mitoxantrone Liposomes + TRAIL

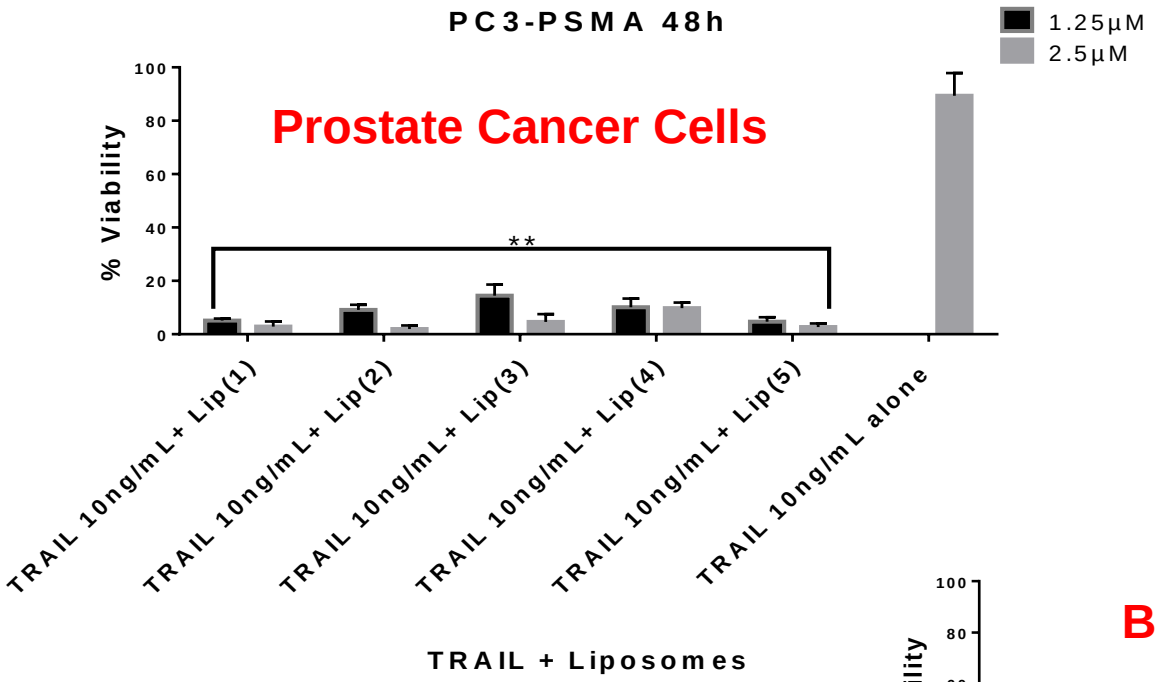


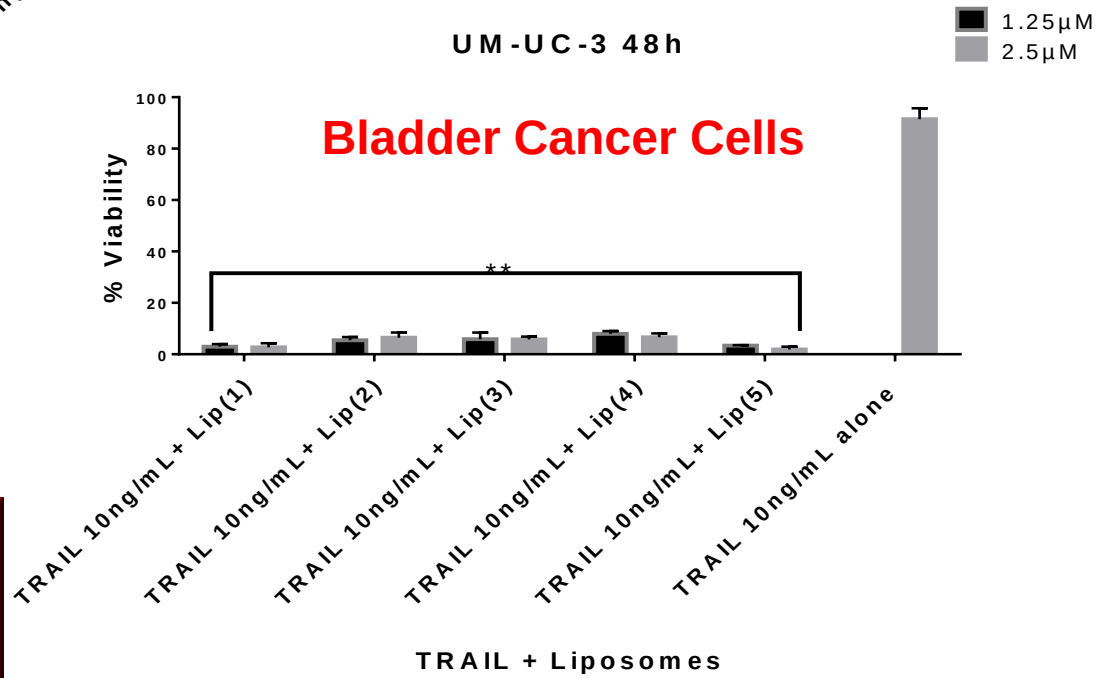
TRAIL alone (indicates cancer cell resistance)

** $p \leq 0.05$, Compared combination of Mitoxantrone containing liposomes + TRAIL vs Free TRAIL

TRAIL + Mitoxantrone-Liposomes are effective for ablation of TNBC Cells

Prostate and Bladder Cancer Cells





Similar results in other cancer cells

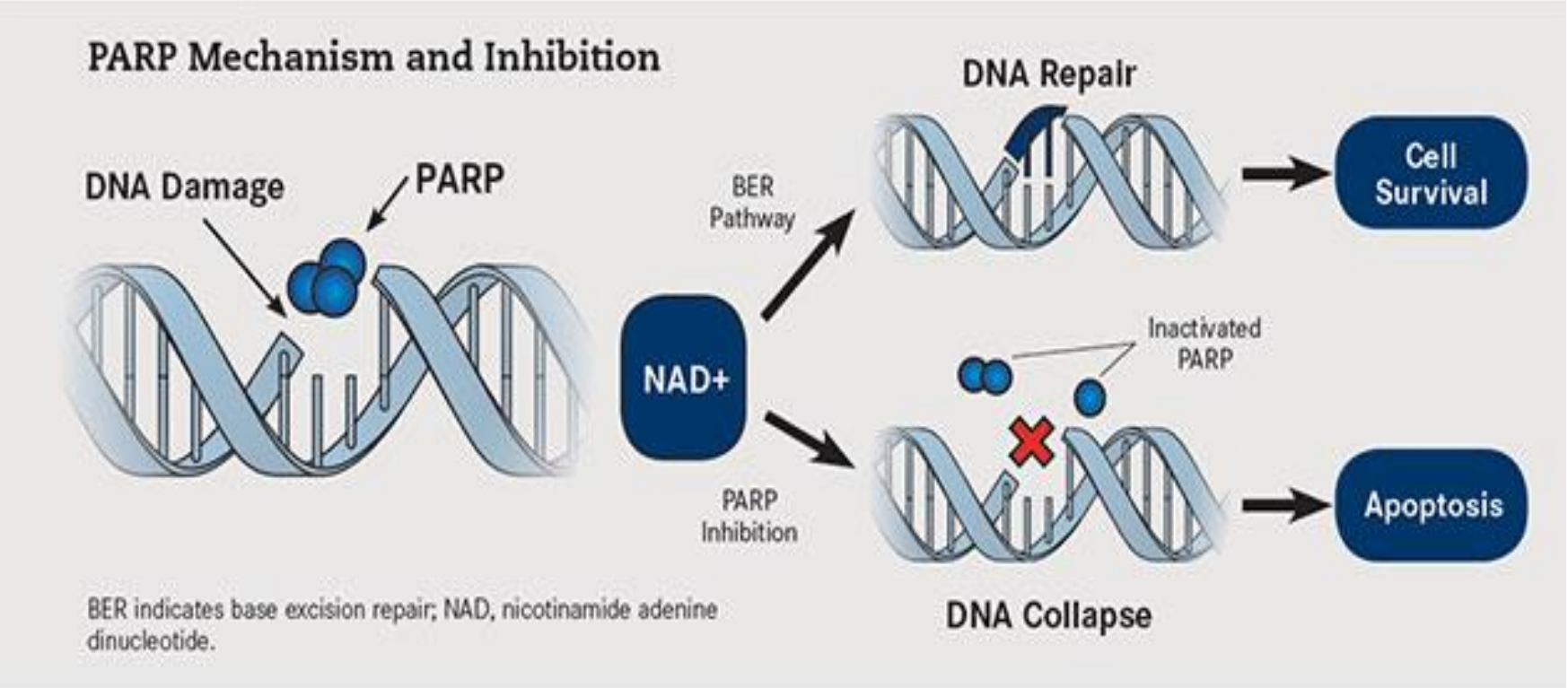
II.

Mitoxantrone-loaded liposomes

+

PARP inhibitors (delivered separately)

PARP Inhibitors



From http://www.onclive.com/publications

Mitoxantrone induces DNA double-strand breaks

PARP is an enzyme that repairs double-strand breaks

Inhibition of PARP + mitoxantrone → enemy's enemy is my friend

PARP Inhibitors (PARPi)

Targeted activity in BRCA-mutated cancers including TNBC

Olaparib

- **FDA approved PARP inhibitor for the treatment of Ovarian cancer**

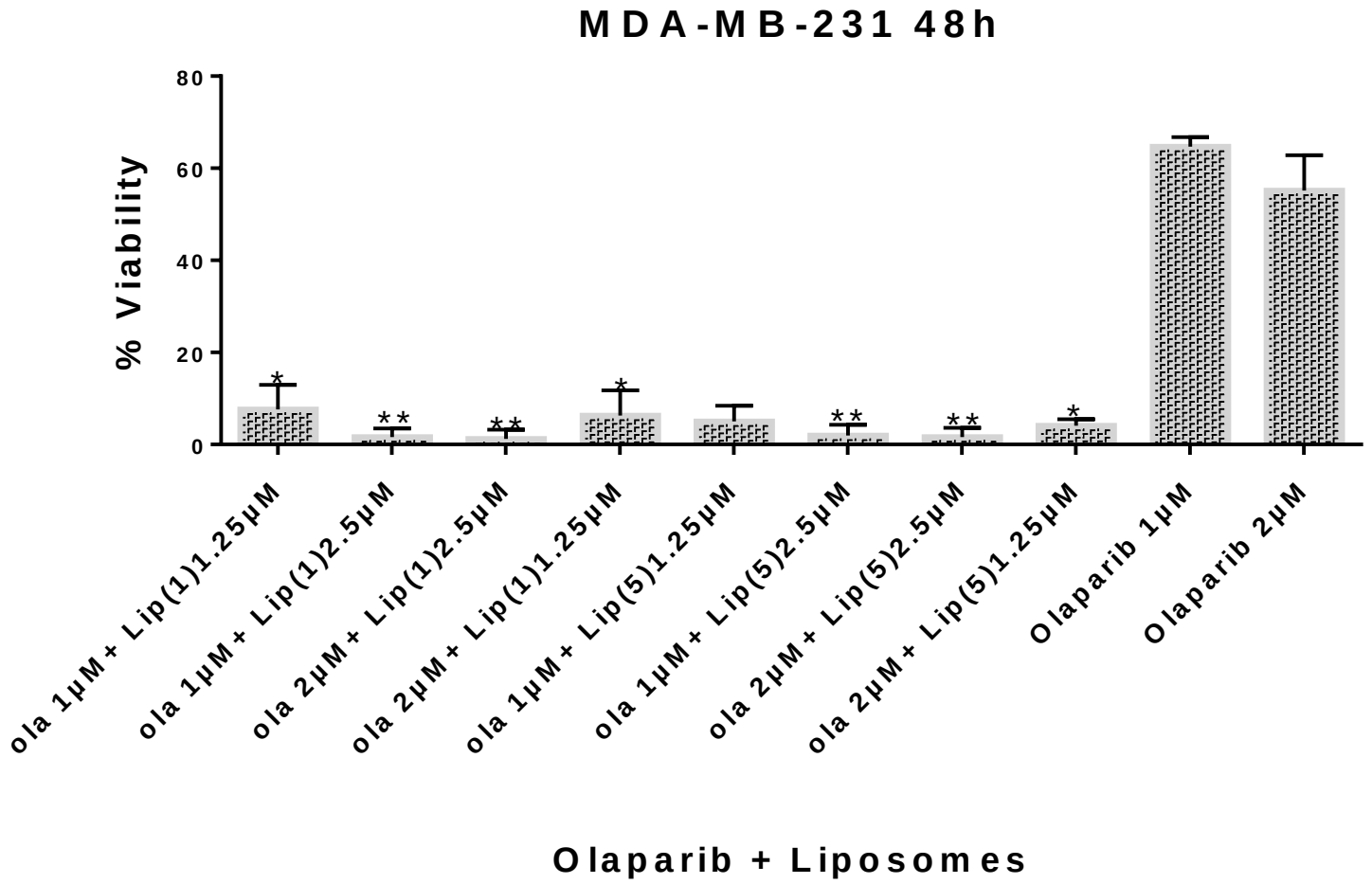
Veliparib

- **Anti-cancer drug for treating metastatic melanoma and breast cancer**

DNA damaging drug: Mitoxantrone

Drug that prevents DNA repair: PARP inhibitors

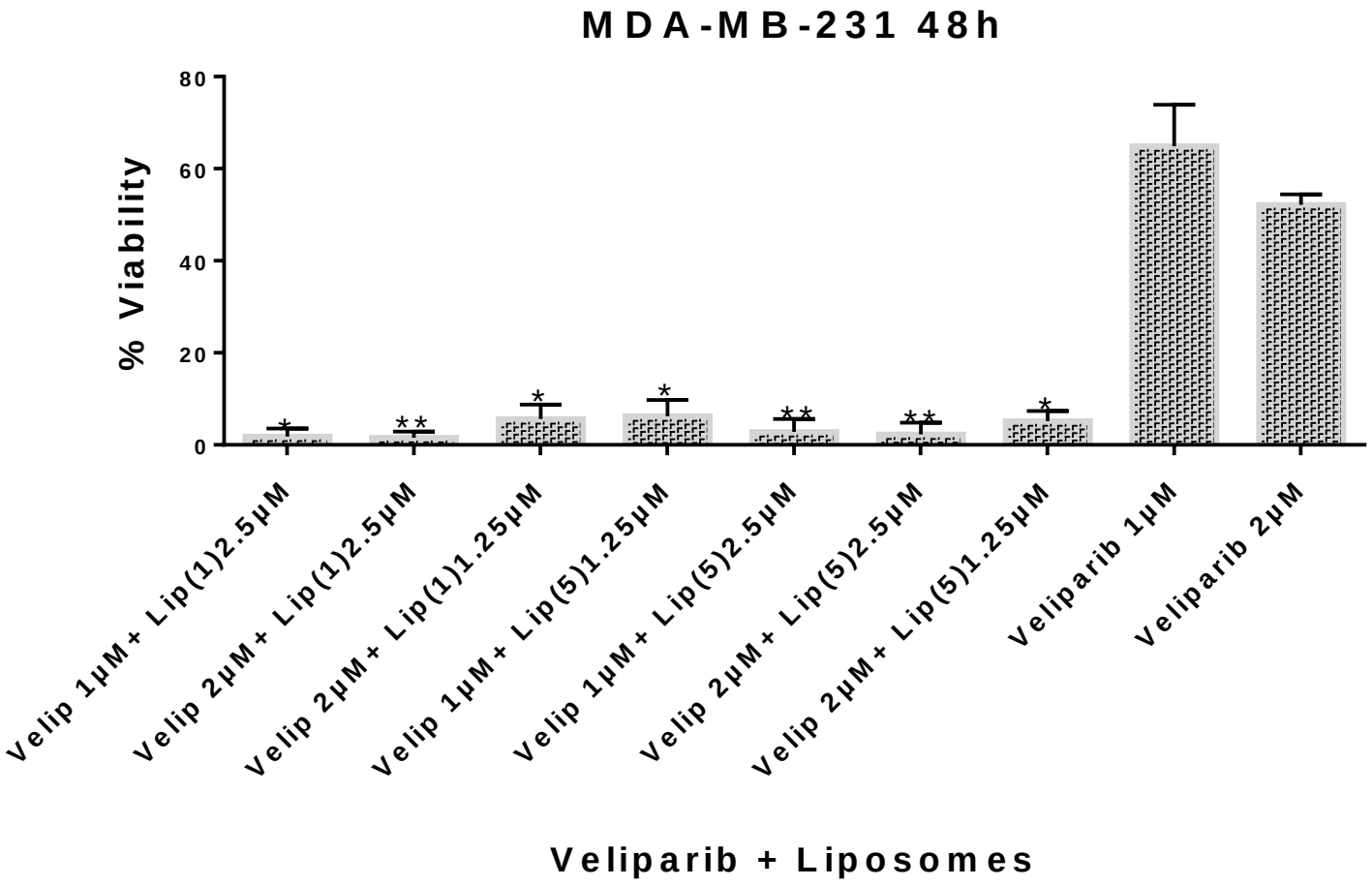
TNBC Cells: Mitoxantrone-Liposomes + Olaparib



** p ≤ 0.05, Compared combination of Mitoxantrone containing liposomes + Olaparib Vs Free Olaparib

Similar results in bladder and prostate cancer cells

TNBC Cells: Mitoxantrone-Liposomes + Veliparib



Near-complete ablation of TNBC cells in culture

Summary & Ongoing Research

- **Synthesis and characterization of mitoxantrone-loaded, aminoglycoside-derived liposomes**
  - Mechanistic studies of mitoxantrone-induced cancer cell death are underway
- **Demonstration of efficacy of mitoxantrone-liposomes and their combinations with TRAIL and PARP inhibitors.**
  - **Additional dose studies underway**
- **Preliminary / Ongoing Studies:**
  - **Folate-conjugated polymers for targeting TNBC cells:** synthesis, characterization, and targeted uptake
  - **Generation of liposomes encapsulating PARP inhibitors**
  - **Establishment of the orthotopic TNBC tumor model in mice (Prof. Gendler) for evaluating effective treatments**

Acknowledgments

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Arizona Biomedical Research Commission (ABRC)

Biomedical Investigator Grant (BIG) in collaboration with Prof. Sandra Gendler, Mayo Clinic, Scottsdale, AZ.

Identification and Functional Characterization of Novel Neuromuscular Disease-Causing Variants in Arizona Infants and Children

Lisa Baumbach-Reardon, Ph.D.

TGen

Arizona Biomedical Research Commission Award
2014-2017

Dr. Lisa Baumbach-Reardon

Education, Training and Experience

- **Ph.D., Biochemistry and Molecular Biology, Univ. of Florida, Gainesville**
- **Postdoctoral Training, Baylor College Of Medicine**
  Thomas Caskey—DNA Diagnostics and genotypic studies of DMD/BMD patients
- **Fellowship in Human Genetics- Univ. of Colorado, Denver**
  Resulted in ABMG Board dual certification in Clinical Molecular Genetics and Biochemical Genetics

Faculty member at University of Miami/Miller School of Medicine – 20 yrs.
Primary Research interests- Neurogenetics, Human Genetics, Rare diseases—which led us to the X-Linked SMA (XL-SMA) Story

2011—Moved to Tgen – start the new Dorrance CLIA Lab and continue our exciting research in XL-SMA and other rare neurogenetic disorders.

ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization

The underlying premise of these studies is that investigating rare diseases will lead to greater understanding of common disease mechanisms

Why Study Neuromuscular Disease?

- There are numerous forms of neuromuscular disease that affect both adults and children
- They are often fatal or debilitating
- There is great phenotypic and genetic variability thus diagnosis is not easy

What is Neuromuscular Disease (NMD)?

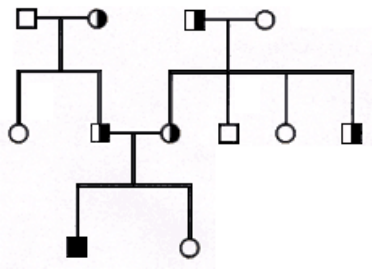
- A neuromuscular disease is a disorder that affects the peripheral nervous system
- Peripheral nervous system includes muscles, the neuromuscular junction, peripheral nerves in the limbs, and the motor-nerve cells in the spinal cord
- Patients with neuromuscular diseases may present with:
  - weakness
  - loss of muscle bulk
  - muscle twitching, cramping
  - numbness, tingling, and other symptoms

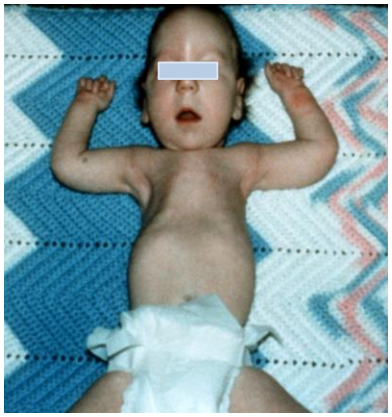
Anterior Horn Cell Disease—2 types

Spinal Muscular Atrophy (SMA)

Features of Disease:

- SMA is an autosomal recessive disorder affecting ~1 in 10,000 live births
- Most common genetic cause of infant mortality
- Mutation in Survival Motor Neuron (SMN) gene results in decreased levels of SMN protein
- Carrier frequency is ~1in 40 to 1 in 60
- Attacks motor neurons which control voluntary muscles
- Anterior horn cells (lower motor neurons) in base of brain & spinal cord gradually degenerate
- Results in muscle weakness and atrophy
- Respiratory failure from diaphragmatic muscle involvement late in the disease





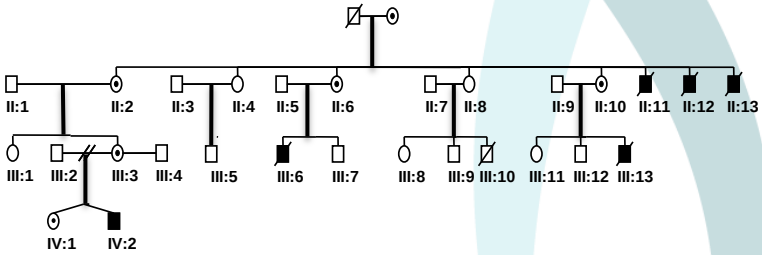
X-linked Spinal Muscular Atrophy (XL-SMA)

Features of Disease:

- X-linked
  - No reported phenotype in female carriers
  - Usually infantile lethal in males
- Severe congenital hypotonia (muscle weakness)
- Contractures / Arthrogryposis (flexed joints)
- Bone fractures at birth (sometimes)

In 2008 we identified *UBA1* mutations as the genetic cause of XL-SMA

Ramser *et al* 2008 Am J Hum Genet 82:188-93



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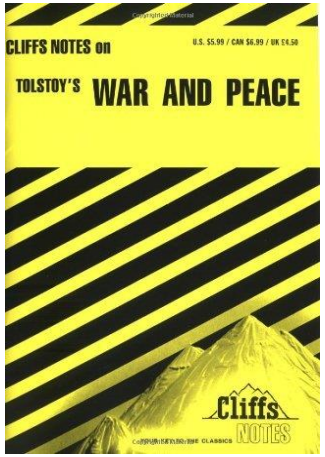
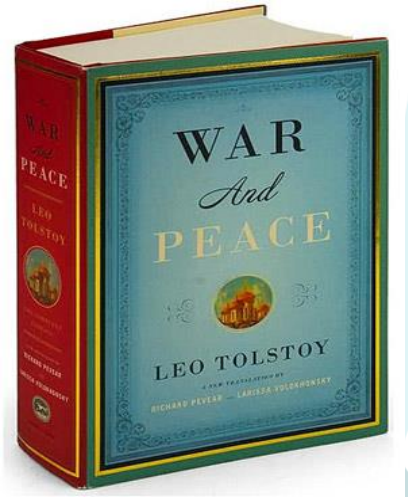
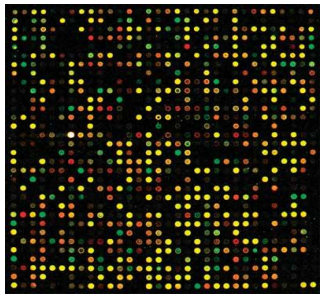
Whole exome sequencing

- Sequence all ~25,000 genes at once
- Use supercomputer to identify changes in the sequence that cause SMA and related neuromuscular disease.

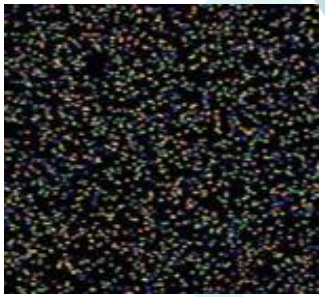




~2000-2005



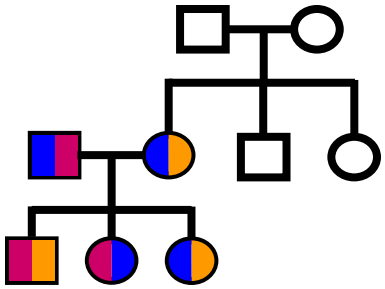
2006-Present



Summary of ABRC exome studies 2014-2016

Families enrolled in exome studies	18
Families with exomes completed	12
Individuals in exome studies	70
Individual exomes completed	45
Affected individuals sequenced	26
Families with mutations identified	9*

*All were clinically confirmed by Sanger sequencing of proband



- Many of the families had only one affected individual
- All the cases had numerous diagnostic tests without receiving a diagnosis prior to exome sequencing.

Nemaline Myopathy
NEB Compound Heterozygous Recessive

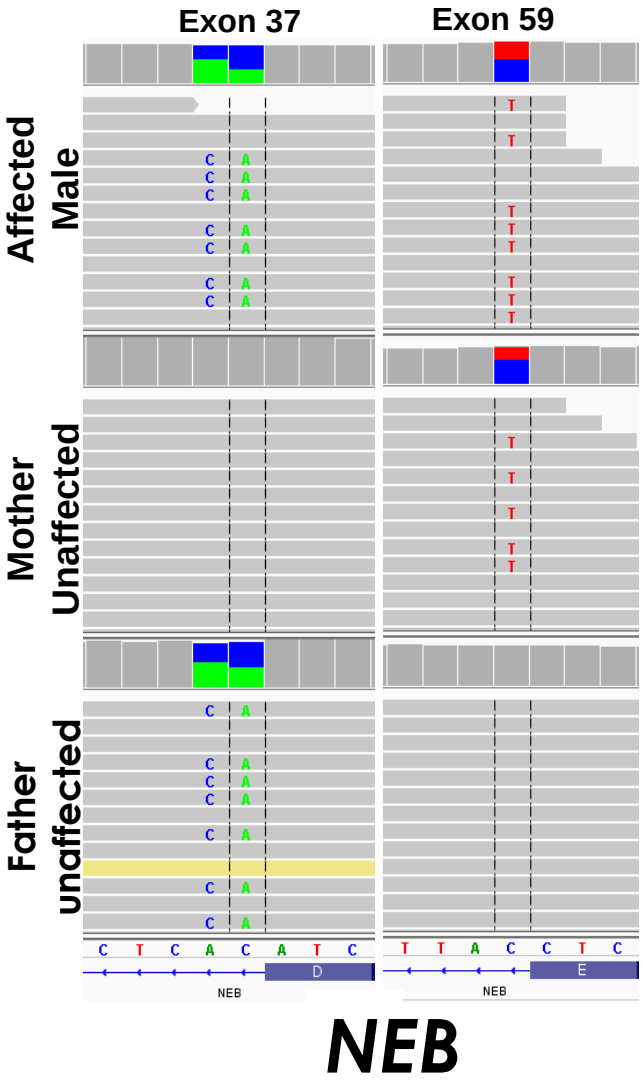
Family 74

Phenotype

- Congenital severe global hypotonia
- Trach/vent dependent
- G-tube dependent
- Arthrogryposis
- Bicuspid aortic valve
- Cryptorchidism
- Underdeveloped lung
- Eventrated diaphragm
- Missing 2 ribs
- Deafness
- Mother has history of miscarriages
- Currently ~11 mo. of age
- Muscle biopsy nemaline bodies and myofibrillary disorganization

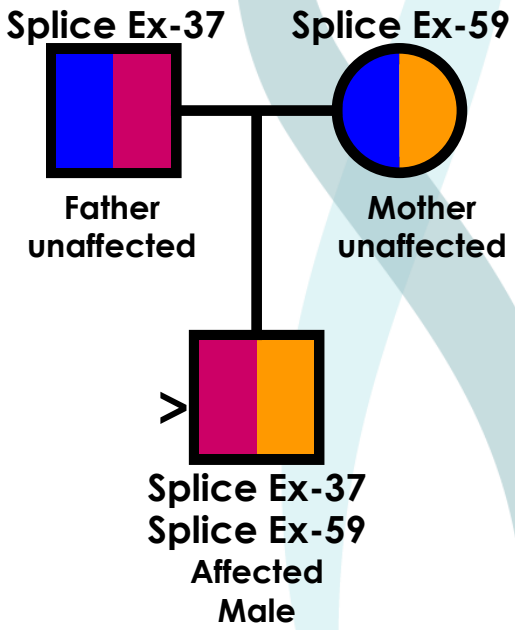
Pathogenic Variants

- Novel splice variant at exon 59 and previously reported pathogenic inversion splice variant at exon 37



Nebulin (NEB)

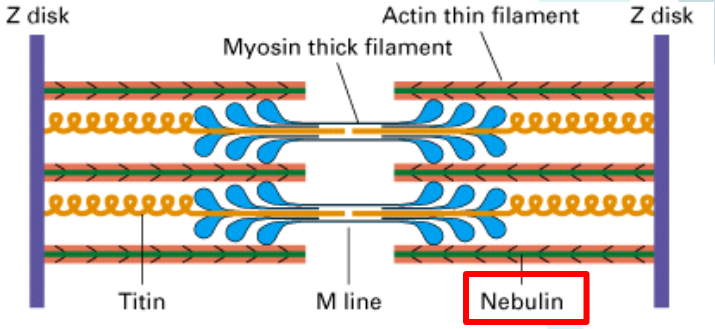
- Essential structural component of muscle that stabilizes actin filaments
- Loss of function mutations in *NEB* cause Nemaline myopathy



NEB – Nebulin

- Critical muscle structural protein; very large protein
- Many missense mutations occur and are benign
- ***Frameshift and splice variants are often pathogenic***
- Cause Nemaline Myopathy
  - Variable phenotypes ranging from early lethality to mild myopathy
  - *Nemaline bodies* are abnormal accumulations of muscle thin-filament proteins caused by mutations in Nebulin (*NEB*) and other genes encoding filament proteins and are characteristic of nemaliine myopathy
  - Titin-nebulin filament system stabilizes alignment of thick and thin filaments in skeletal muscle

To Date we have identified 3 unrelated Arizona families with NEB mutations.



Infantile Neuroaxonal Dystrophy
PLA2G6 Compound Heterozygous Recessive

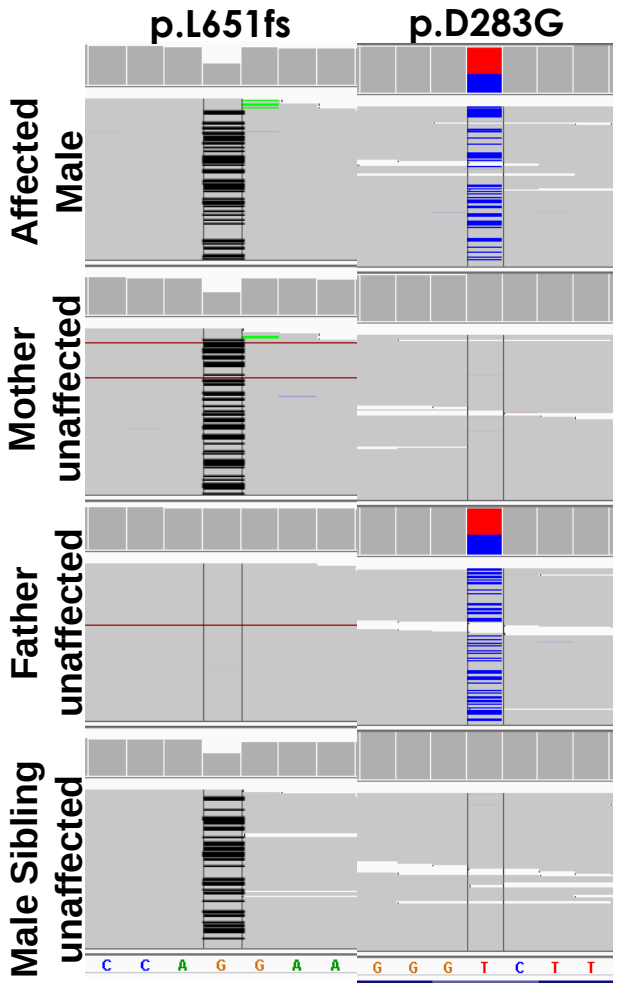
Family 79

Phenotype

* Global neuromuscular delay
* Crawled but regressed
* Progressive cerebellar atrophy
* Generalized hypotonia
* Mild spine deformation
* Bilateral coxa valga
* Weight loss
* Normal CPK
* Mild microcephaly
* Onset ~9mo. of age
* Currently ~3yrs of age

Pathogenic Variants

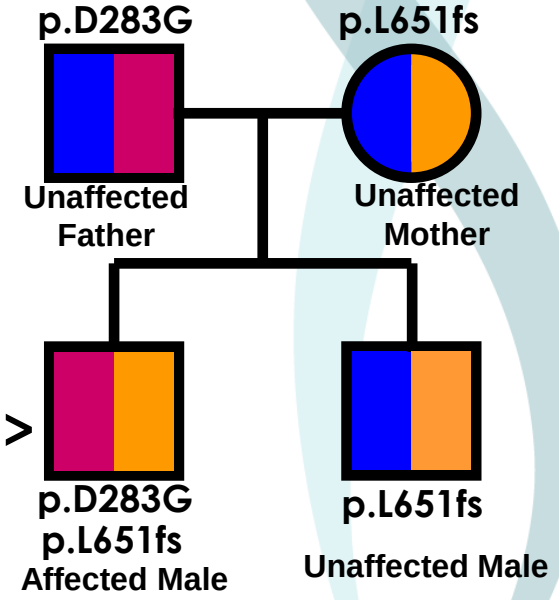
* Novel p.D283G missense and pL651fs. Not present in ExAC



PLA2G6

PLA2G6

* Calcium-independent phospholipase (A2 group VIA)
* essential for membrane phospholipid remodeling in axons and synapses
* Mutations in *PLA2G6* cause Infantile neuroaxonal dystrophy, a very rare disorder



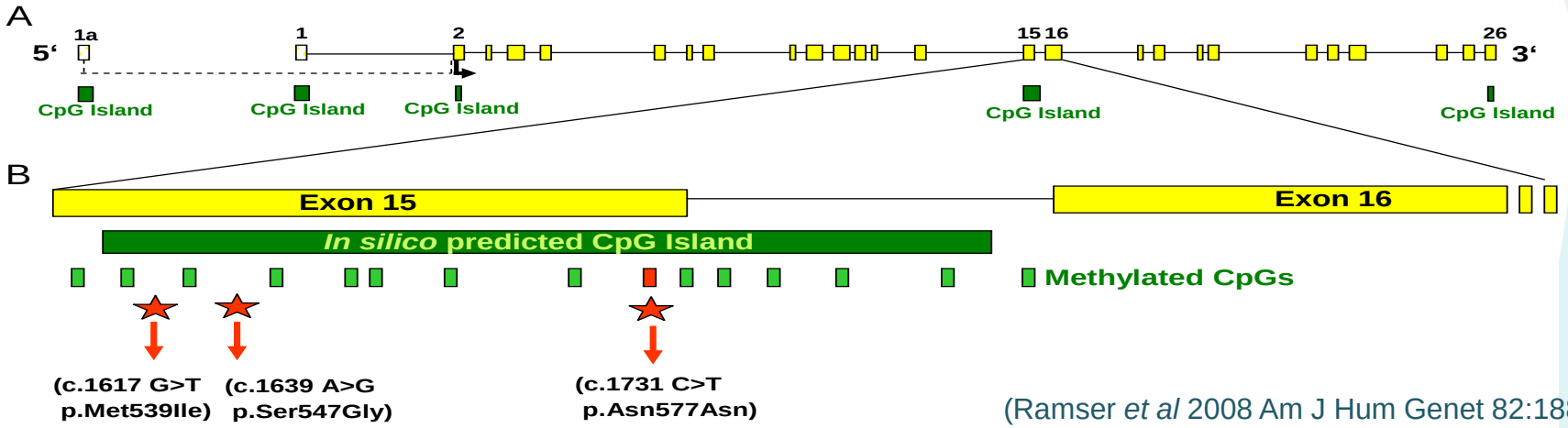
ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

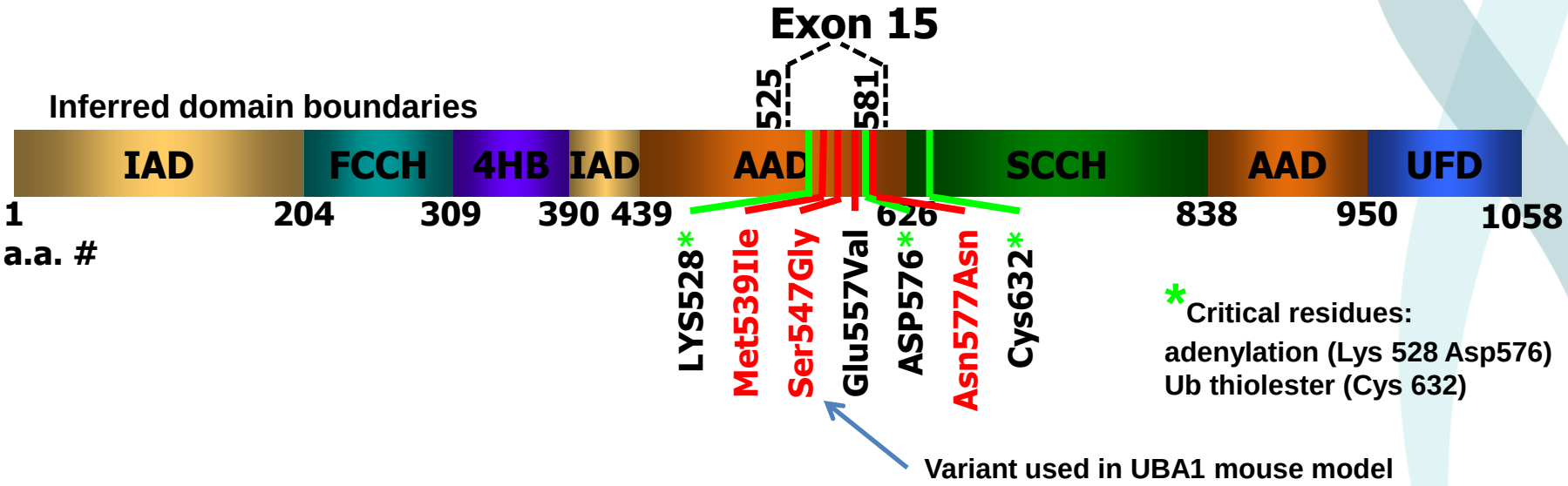
Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization

UBA1 Mutations

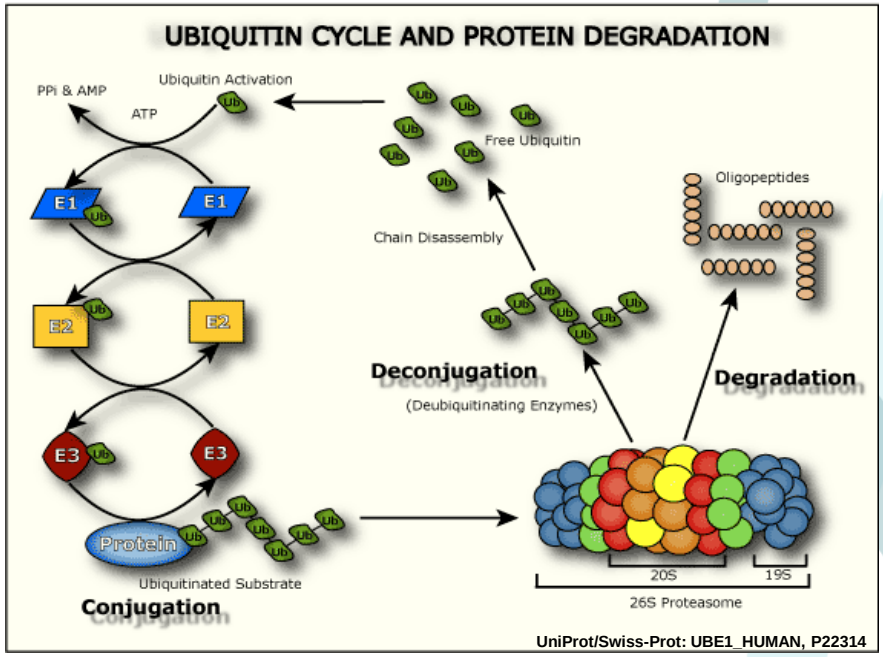


(Ramser *et al* 2008 Am J Hum Genet 82:188-93)



Why is *UBA1* Important?
Ubiquitin Proteasome System

- UBA1 is the initiating pinnacle enzyme in the Ubiquitin Proteasome System (UPS)
- UBA1 is expressed in every cell with highest expression in the spinal cord
- The UPS is responsible for the degradation of most proteins
- Uses Ubiquitin as a death tag, targeting other proteins for destruction via the proteasome
- **Complete loss of *UBA1* function is lethal**

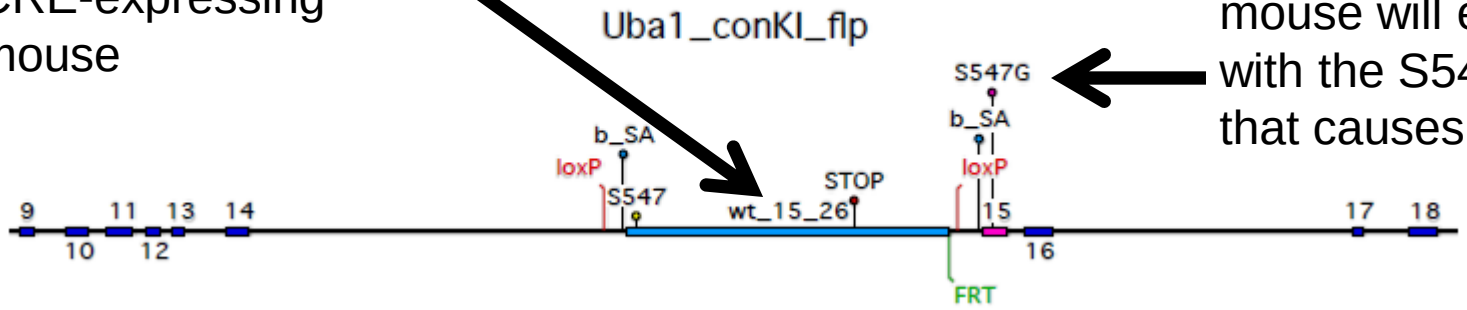


UBA1 Conditional Targeted Mouse Design

The endogenous mouse exon 15 contains the ***UBA1* S547G** mutation but it *will not be* expressed until bred with CRE-expressing mouse

1. This partial mini-gene will express wt *UBA1* until removed by breeding with a CRE-expressing mouse

2. After breeding with CRE-expressing mouse the wt partial minigene will be removed and the mouse will express *UBA1* with the S547G mutation that causes XL-SMA



We have Southern blot confirmation of heterozygous female mice with the conditional targeted knock-in allele (*see poster*).

Initial development of the UBA1 mouse model was made possible by a *Flinn Foundation Grant* and the *ARBC*

Investigate Mechanisms of Disease in UBA1 Mouse Model

UBA1^{S547G} mouse model expansion and survival

We will first test whether the *UBA1*$^{S547G/y}$ hemizygous (male) mouse mutants have similar perinatal lethality as observed in humans

Neuromuscular development in UBA1^{S547G} mutant mice

We will evaluate whether *UBA1*$^{S547G/y}$ mutant mice exhibit aberrant development or degeneration of the neuromuscular system.

Spinal motor neuron number, axonal outgrowth, and NMJ formation will be evaluated at distinct stages of development using immunolabeling, motor neuron specific reporter mice, and microscopy.

Examples of Immunolabeling of motor neurons in normal mice

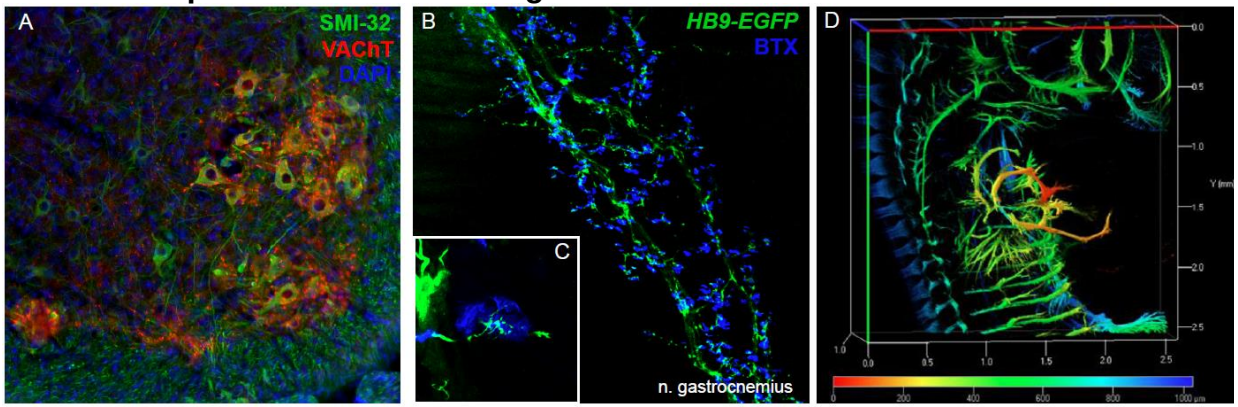


Image courtesy of J. Newbern ASU collaborator

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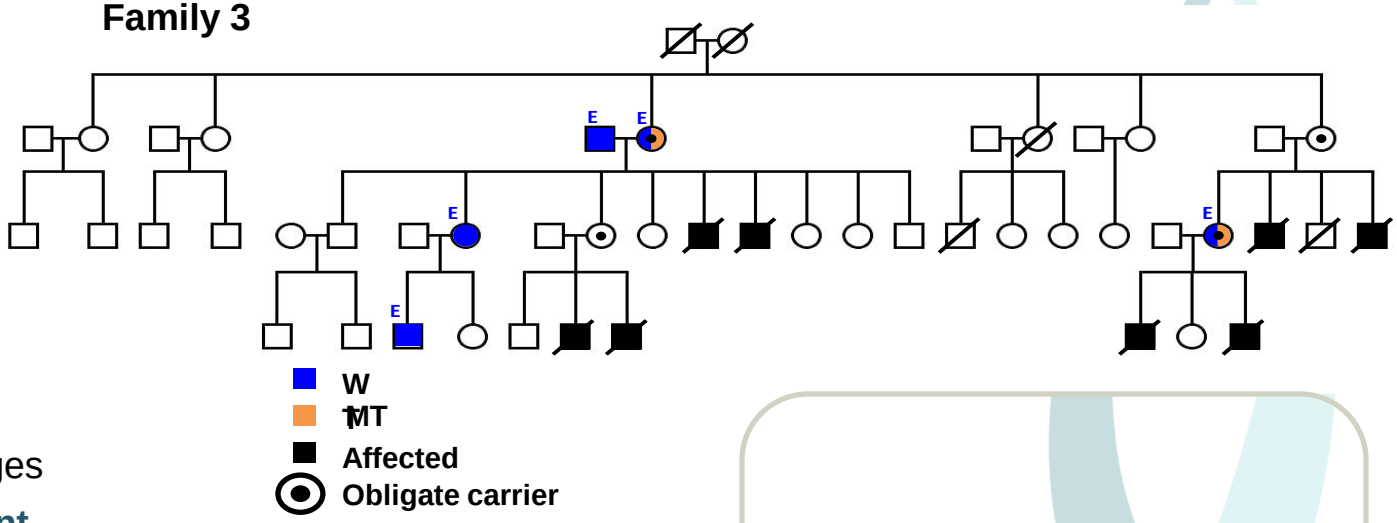
SCML2 Mutation Families

Family 3

Phenotype (Neonatal Males)

* Clear X-linkage
* Neonatal lethality
* Dysmorphic facies
* Arthrogryposis
* Microcephaly
* Hypospadias
* Distal limb deformities
* Subarachnoid hemorrhages



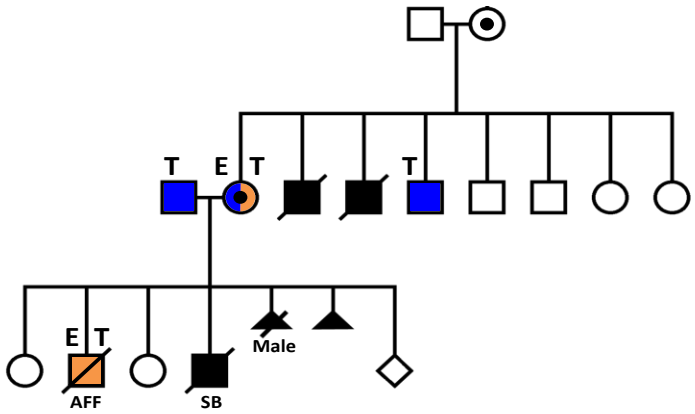
Potential Pathogenic Variant

* **Novel N76S missense in SCML2**

Family 9

Phenotype (Neonatal Males)

* Clear X-linkage
* Neonatal lethality
* Arthrogryposis
* Cerebellar hypoplasia
* Cardiac anomalies



Pathogenic Variant

* **Novel M1V start loss in SCML2 in obligate carrier and affected son**

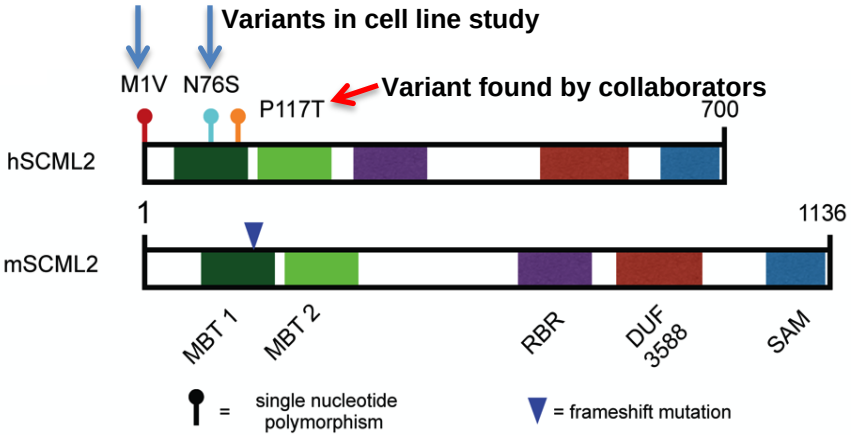
SCML2

* **Never reported as NMD gene**
* **Key component of polycomb repressor complex (2)**
* **Maintains repression of developmental genes**

SCML2 Mutation Functional Study: Mouse Model

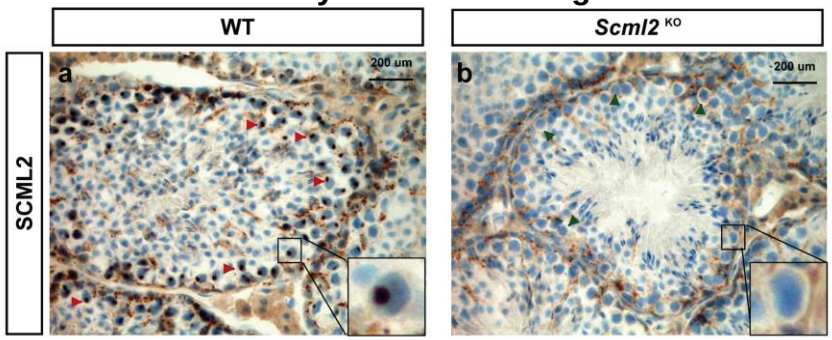
We have developed a collaboration with investigators in Italy, Switzerland, and Germany to study *SCML2* mutations.

- A knock out mouse model with an 11bp frame sh mutation in *SCML2* exon 4 resulting in premature stop codon in the MBT1 domain was generated (*confirmed by Sanger Sequencing*)
- *SCML2*^{KO} males have fertility issues from hypogonadism and spermatogeneis defects
- Crossing wild type males with *SCML2*$^{+/-}$ females produced *SCML2*^{KO} males for study



- Mice exhibited:
  - Impaired inhibitory synapse in spinal motor neuron
  - Impairment of inhibitory currents & synapses
  - Spontaneous cortical hyperexitabilty
  - Susceptibility to seizures

Knock out of Scml2 was confirmed by immunostaining



Portions of these studies are in revision /review at Nature Neuroscience

ABRC Project Overview

Aim 1. Identification of the genetic causes of neuromuscular disease in Arizona infants and children by whole exome sequencing

Aim 2. Characterization of neuromuscular development in an *UBA1* mouse model of XL-SMA

Aim 3. Functional characterization of a novel neuromuscular disease-causing genetic variant

The study of rare diseases provides greater insights into mechanisms which may be relevant to more common related diseases

XL-SMA Working Group 2013

Special thanks to Jesse Hunter, Chris Balak, and Mary Ellen Ahearn (Dr. Saunder Bernes, Phoenix Children's Hospital-not pictured)

ACKNOWLEDGEMENTS

An Integrative Personalized Professional Practice using Mobile Technologies for Weight Management

David Jackemeyer, Yulia Abidov, Karen Herbst, NJ Tao, Craig Stump, Erica Forzani**

eforzani@asu.edu,

ASU ARIZONA STATE UNIVERSITY

BRIDGING THE GAP

Research World

Data Analysis

Real World

Medical and tracking devices

THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE

ARIZONA STATE UNIVERSITY

Diabetes Research Program

BRIDGING THE GAP

Research World

Data Analysis

Real World

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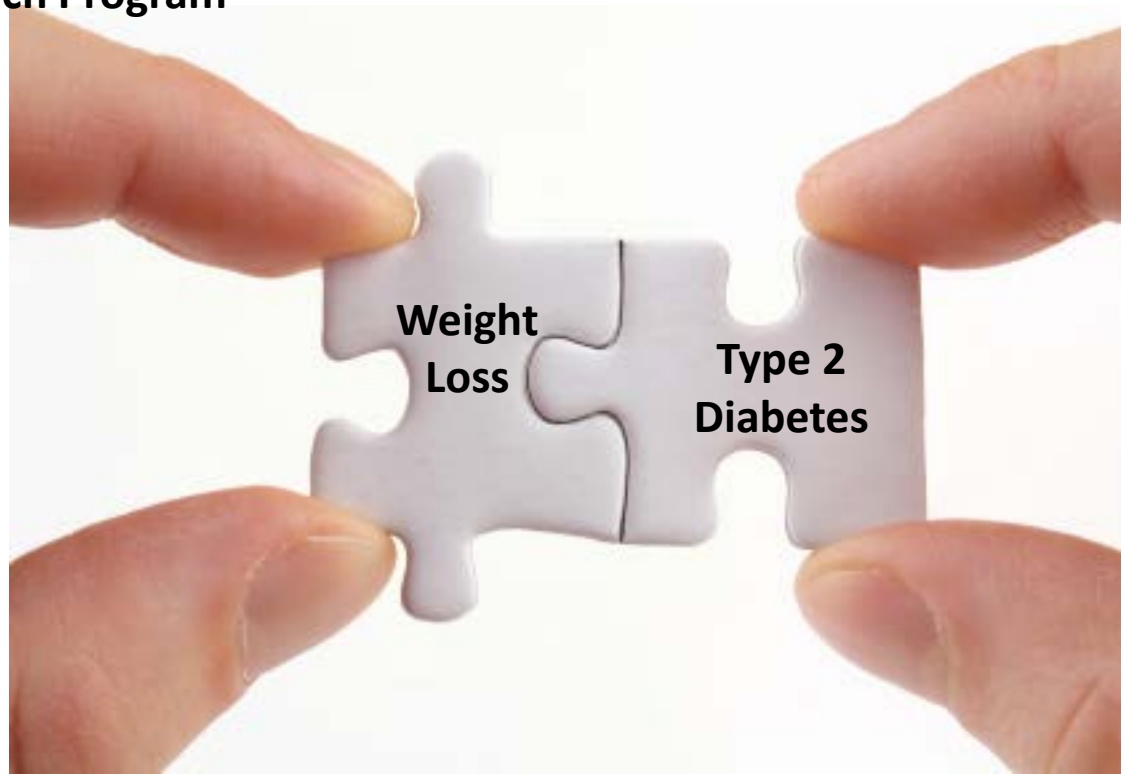
THE UNIVERSITY OF ARIZONA | COLLEGE OF MEDICINE

Diabetes & Weight Loss

Diabetes Research Program

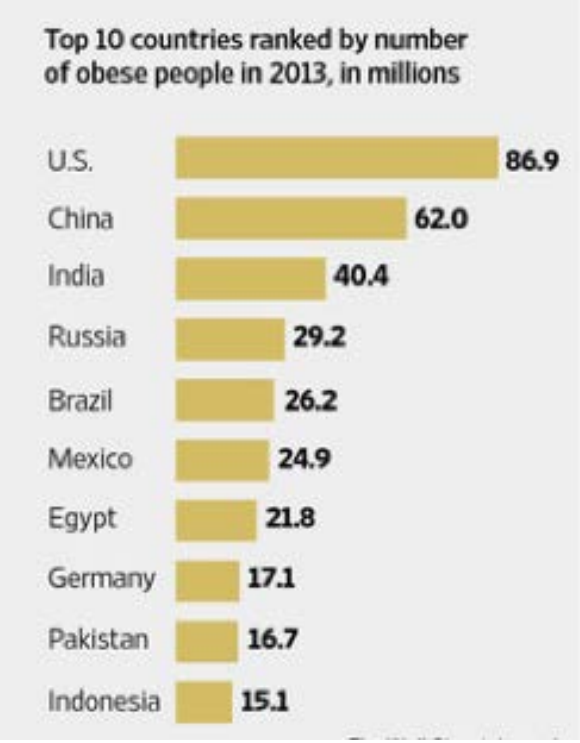


"No matter how heavy you are, you will significantly lower your blood sugar if you lose some weight"

Cathy Nonas, MS, RD
Spokeswoman for the American Dietetic Association
Professor at Mount Sinai School of Medicine, NY

The Motivation

- 2.1 billion people, or ~30% world's population, are overweight or obese in 2013
- Obesity is known to cause many chronic diseases, including heart diseases, stroke, diabetes, metabolism syndrome, and some cancers (CDC).
- People spend ~$600 billion per year, yet most are frustrated with the results



2 out 3 Americans are overweight or Obese

The Problem

- Most people know weight management requires balanced diet and exercises, but few know:

How much should I eat?

How much should I exercise?

Why is my weight like yo-yo?

Energy Conservation Law

Caloric Intake *Weight* *Total energy expenditure (TEE)*

=

+

[

+

]

Resting (REE or RMR) Physical Activity

~ 80-90% < 20 % (sedentary)

Antoine Lavoisier
1743-1794

Energy Conservation Law

Weight

Caloric Intake

=

-

Total energy expenditure (TEE)

+

Resting (REE or RMR)

~ 80-90%

Physical Activity

< 20 % (sedentary)

Affordable & Mobile Technologies

Affordable & Mobile Technologies

Importance of REE or RMR in weight management

Position of the American Dietetic Association: Weight Management

This paper endorsed by the American College of Sports Medicine

J Am Diet Assoc. 2009;109:330-346.

EAL Recommendation "Estimated energy needs should be based on [resting metabolic rate]. If possible, [resting metabolic rate] should be measured (eg, indirect calorimetry).

Rating: Strong

Resting Metabolic Rate (**RMR**) = "Metabolism" = Resting Energy Expenditure (**REE**)

H. Seagle, G. W. Strain, A. Makris, and R. S. Reeves, "Position of the American Dietetic Association: Weight Management," Journal of the American Dietetic Association, vol. 109, pp. 330-346, 2009.

Energy Conservation Law

Total energy expenditure (TEE)

Weight = ***Caloric Intake*** - [Resting (REE or RMR) + Physical Activity]

~ 80-90%

< 20 % (sedentary)

Affordable & Mobile Technologies

Recent tool in the market

Affordable & Mobile Technologies

Energy Conservation Law

Total energy expenditure (TEE)

Weight

Caloric Intake

=

-

Resting (REE or RMR)

~ 80-90%

+

Physical Activity

< 20 % (sedentary)

Affordable & Mobile Technologies

Recent tool in the market

Affordable & Mobile Technologies

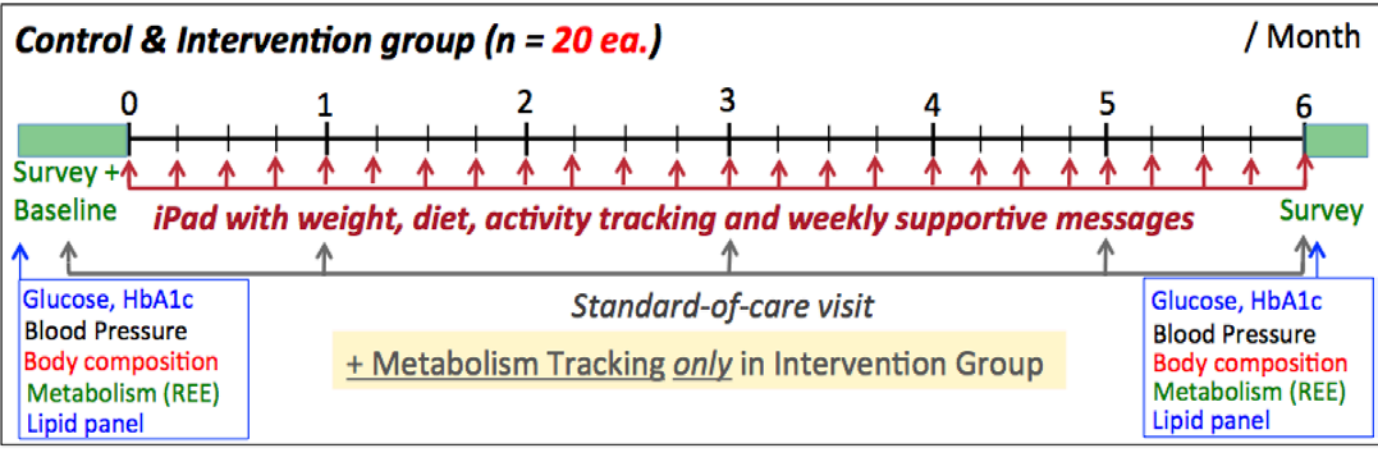
Six-month study design

Control Group (CG)

Intervention Group (IG)

•The participants from the control group had an iPad with My Fitness Pal App to track calorie intake, an activity tracker to track steps and floors, and a weight scale.

• Each participant in the control group was recommended a 500-calorie deficit intake based on the Harris Benedict Equation



Baseline: initial measurement period; **Surveys:** subjects pre- and post study survey, Metabolism tracker: Breezing

• The intervention group had the same gadgets as the control group, as well as a Breezing Tracker.

• Both groups were followed up with a Standard-of-Care procedure for 6 months, and were reached by e-mail every 2-3 weeks with general health information.

** Most of participants had T2 Diabetes, or were at risk of Diabetes*

Clinical study in an overweight and obese population*

Dr. Craig Stump, MD

Characteristics of the population

FLINN FOUNDATION | THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE

Table 1. Physical characteristics of recruited study participants. Means +/- (SD)

Physical Parameters	Age	Weight (kg)	Height (m)	BMI (kg/m^2)	W/H	Fat%	Sys BP	Dias BP
CG (n=20) F:14, M:6	54 (7)	102 (20)	1.68 (0.08)	36 (6)	0.88 (0.10)	44 (8)	127 (14)	81 (7)
IG (n=20) F:17, M:3	57 (13)	92 (14)	1.64 (0.10)	34 (6)	0.85 (0.06)	44 (6)	132 (20)	85 (14)
Normal range	N/A	N/A	N/A	18.5–24.9	N/A	N/A	N/A	N/A

BMI: body mass index,, Waist to hip ratio: W/H ratio, Body fat percentage: Fat%, Blood pressure: BP, Sys BP: systolic BP, Dias BP: diastolic BP.

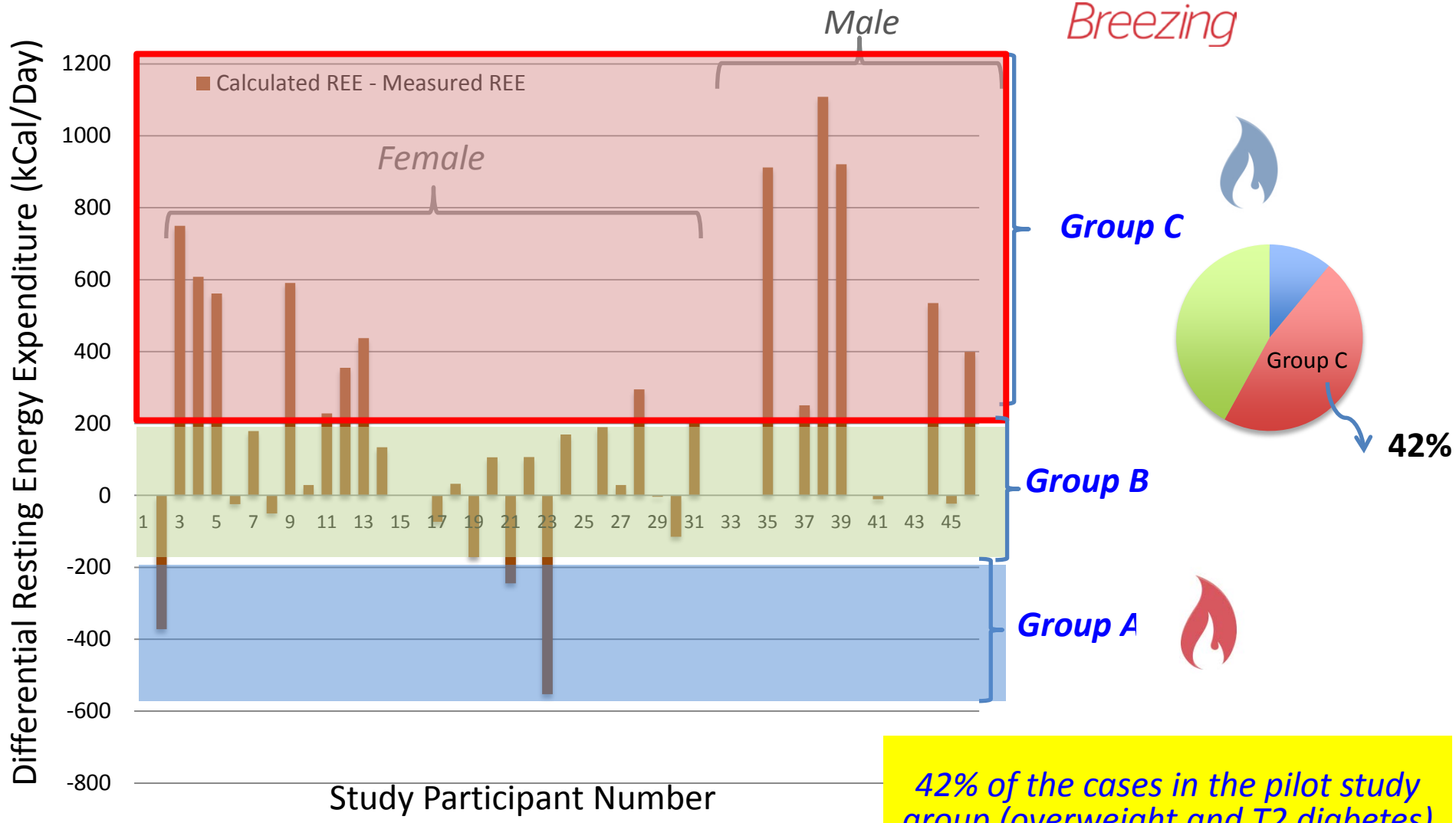
Table 2. Metabolic and blood parameters of recruited study participants. Means +/- (SD)

Parameter	REE (kCal/d)	Gluc. (mg/dL)	Glyc. Hb (%)	Trigly. (mg/dL)	Chol. (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	LDL/HDL	DHRI
CG (n=20) F:14, M:6	1420 (300)	109 (33)	6.6 (1.1)	148 (65)	208 (33)	130 (37)	52 (11)	2.8 (1.3)	6/11= 54%
IG (n=20) F:17, M:3	1570 (280)	111 (27)	6.7 (1.5)	120 (42)	200 (36)	130 (37)	51 (9)	2.7 (1.0)	7/12= 58%
Normal range	N/A	70-105	<6.0	0-169	0-200	0-99	>38	1.3-4.7	

REE: resting energy expenditure, Gluc.: glucose, Glyc Hb: glycosylated Hemoglobin, Trigly.: trygliceride, Chol: cholesterol., DHRI: Diabetes High Risk Index, percentage a new cases discovered with Glyc Hb levels higher than 6.0%.

** Most of participants had T2 Diabetes, or were at risk of Diabetes*

Difference of Calculated REE* – True (measured) REE



* Predictive Equation (Harris-Benedict)

42% of the cases in the pilot study group (overweight and T2 diabetes) had slower metabolic rates that the predicted from equation

Dr. Craig Stump, MD

THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE FLINN FOUNDATION

Why track metabolism?

1700 kCal/day

Oxygen

Carbon Dioxide

1200 kCal/day

Oxygen

Carbon Dioxide

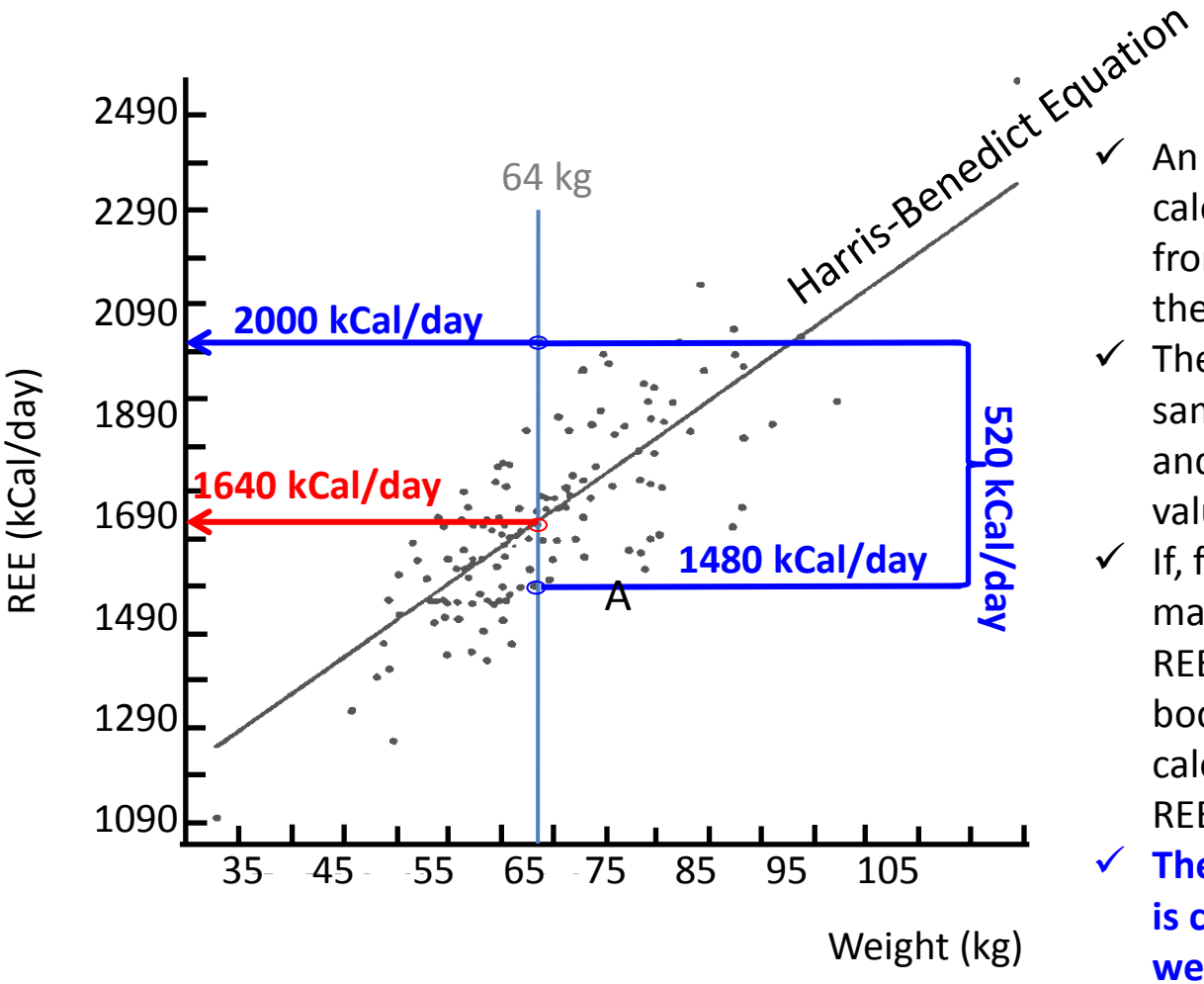
Weight: 112 lbs
Height: 5’ 6’’
Age: 29 years

Your metabolism = Your energy = Your Fire

Different people have different metabolisms

Why we can't use equations to calculate REE ?

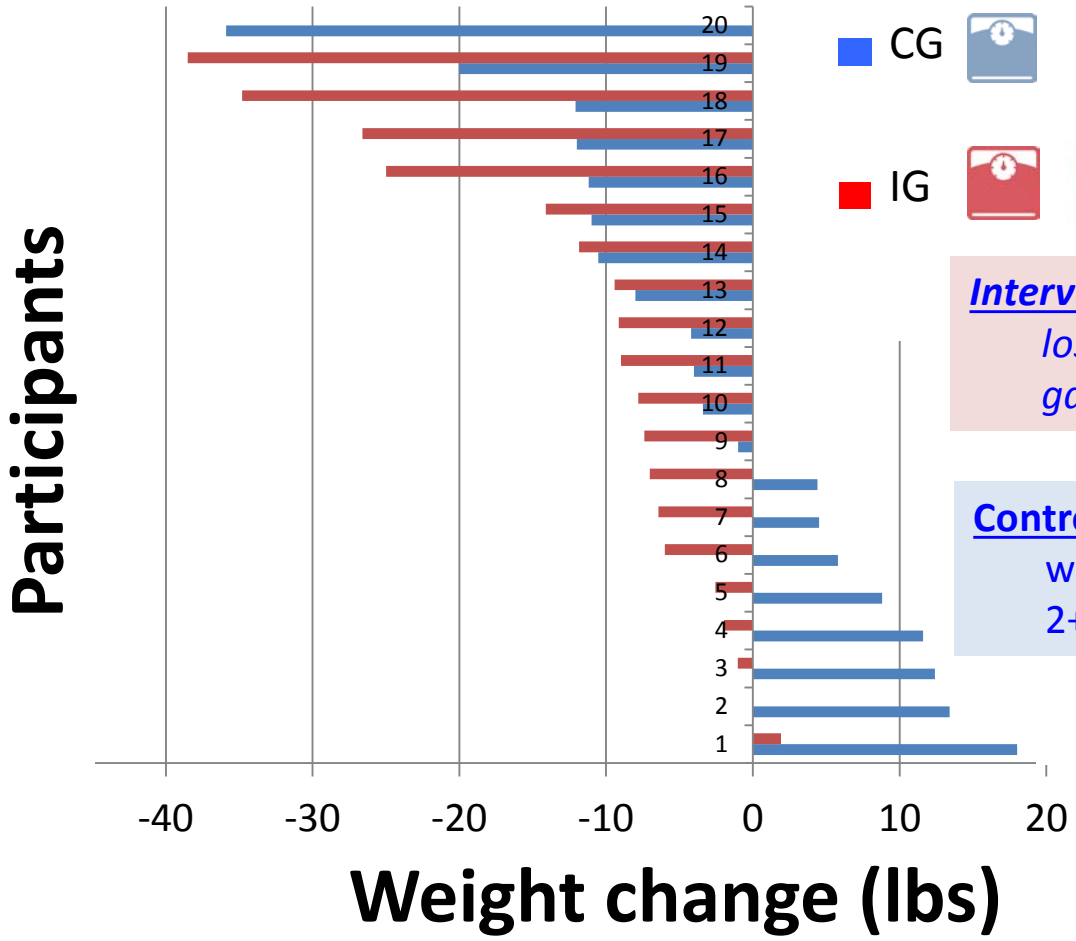
Data from seminal Harris-Benedict's work



- ✓ An actual REE value (from indirect calorimetry measurement) can differ from an estimated REE value (from the *Harris-Benedict* calculation).
- ✓ The results show that for people of same gender and weight (e.g. men and 63 kg) the difference in actual REE values can be as high as 520 kCal/day.
- ✓ If, for instance, subject A's goal is to maintain weight, and the estimated REE (1640 kcal/day) is higher than the body's actual REE (1480 kcal/day), a calorie recommendation based on the REE estimate will lead to weight gain.
- ✓ **Therefore, accurately *measuring* REE is crucial in establishing an effective weight management plan.**

Plot from J. Arthur Harris and Francis G. Benedict, A Biometric Study of Human Basal Metabolism, Proc Natl Acad Sci U S A. 1918 December; 4(12): 370–373.
Criscione, L. & Durr-Gross, M. Eating healthy and dying obese. *Vitasanas GmbH,* http://www.vitasanas.ch, *ISBN: 978-3-0033-02225-6* (2010).

Weight changes



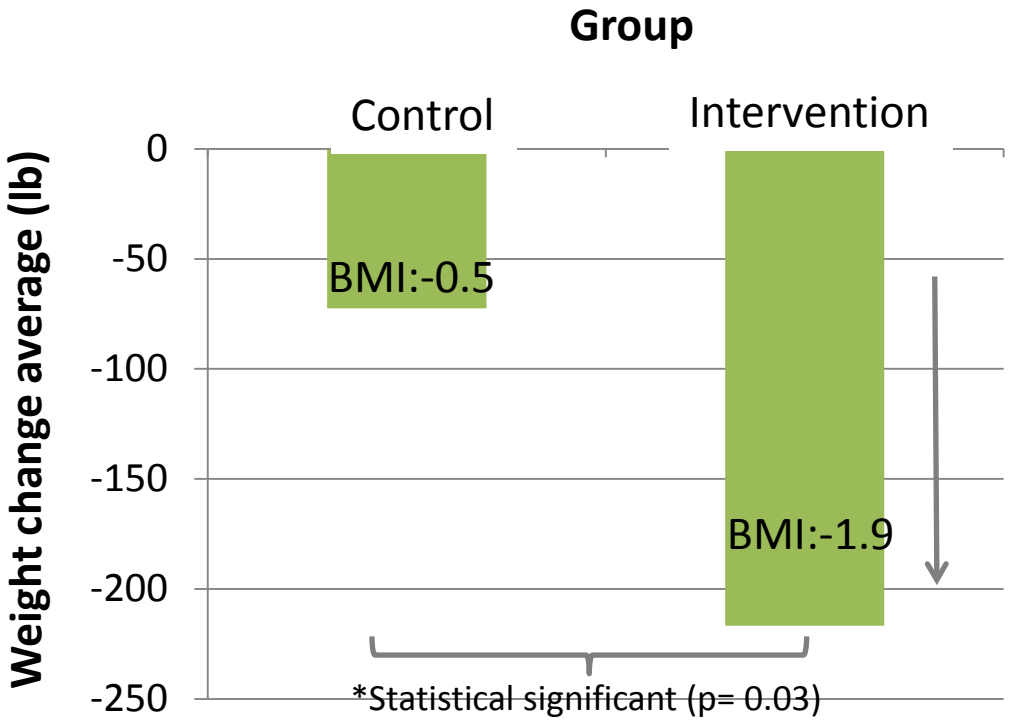
Intervention Group: *17 of 19 participants (**89%**) lost weight, 1 stayed steady and 1 (5%) gained 1.9 lbs.*

Control Group: 11 of 20 participants (**55%**) lost weight, 1 stayed steady and 8 (**40%**) gained 2+ lbs.

Other results:
Weight loss Greater Than 6 lbs:
*CG: **40%** (8/20) **vs** IG: **68%** (13/19)*

Observation: Weight change is accounted from 1st day the participant use MFP (baseline period) up to 6 months after the study

Case #2: Weight & Body Mass Index (BMI) changes



The Intervention group's total weight loss 3x's Greater than control group

The difference in BMI changes in intervention group was statistically significantly different with respect with control group

Intervention group's drop of BMI from 35.5 resulted in change from Obese Class II Group to Obese Class I Group

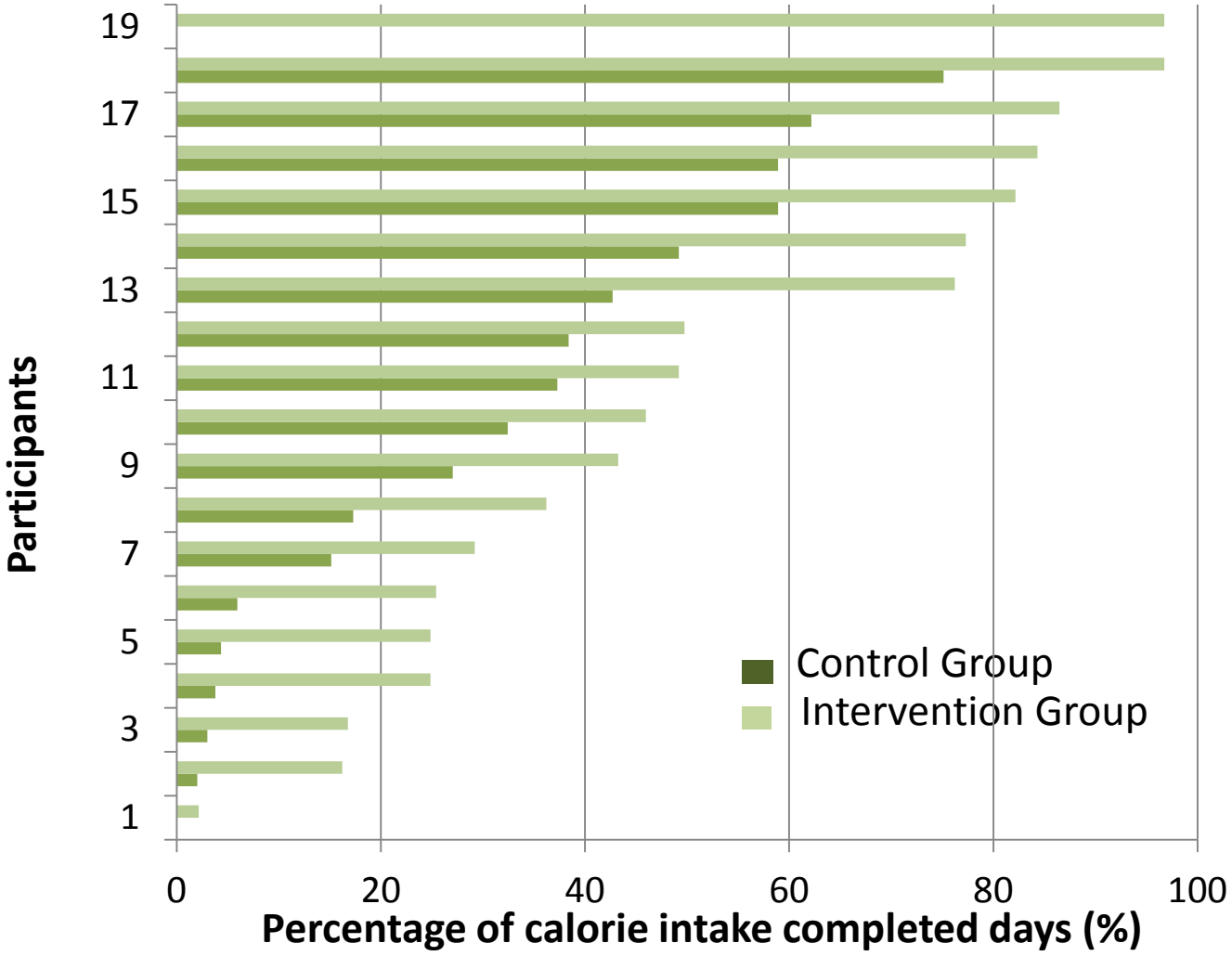
Control group's drop of BMI from 36.9 was not large enough to move out of Obese Class II Group

Unpaired *t* test results

P value and statistical significance:
The two-tailed P value equals 0.0296
By conventional criteria, this difference is considered to be statistically significant.

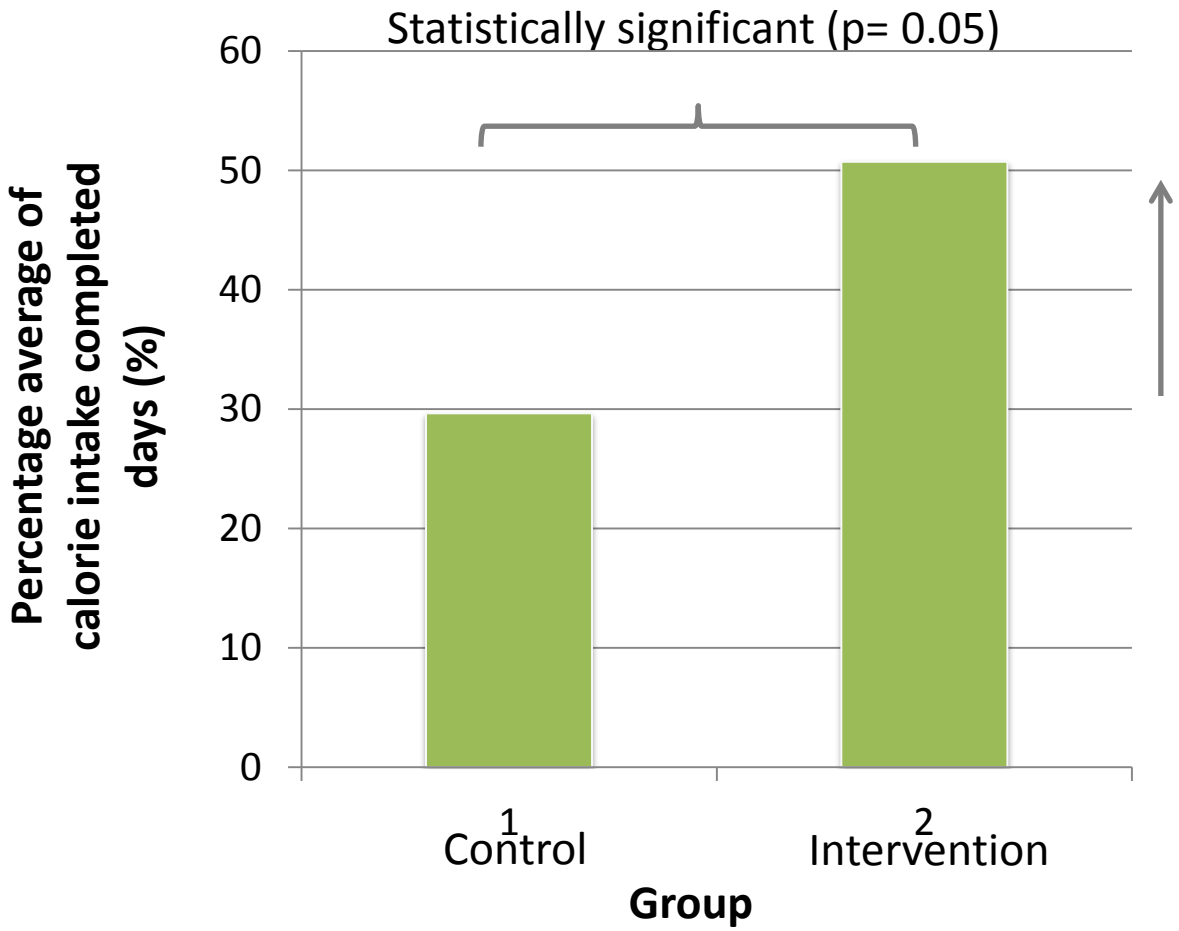
Group	Group One	Group Two
Mean	-0.5023848000	-1.9214530000
SD	1.9203068900	1.9965590000
SEM	0.4293936744	0.4580420482
N	20	19

Case #2: Calorie Intake Completed Days*



* Completed days represent calorie intake values with equal or 25%+ of recommended calorie intake

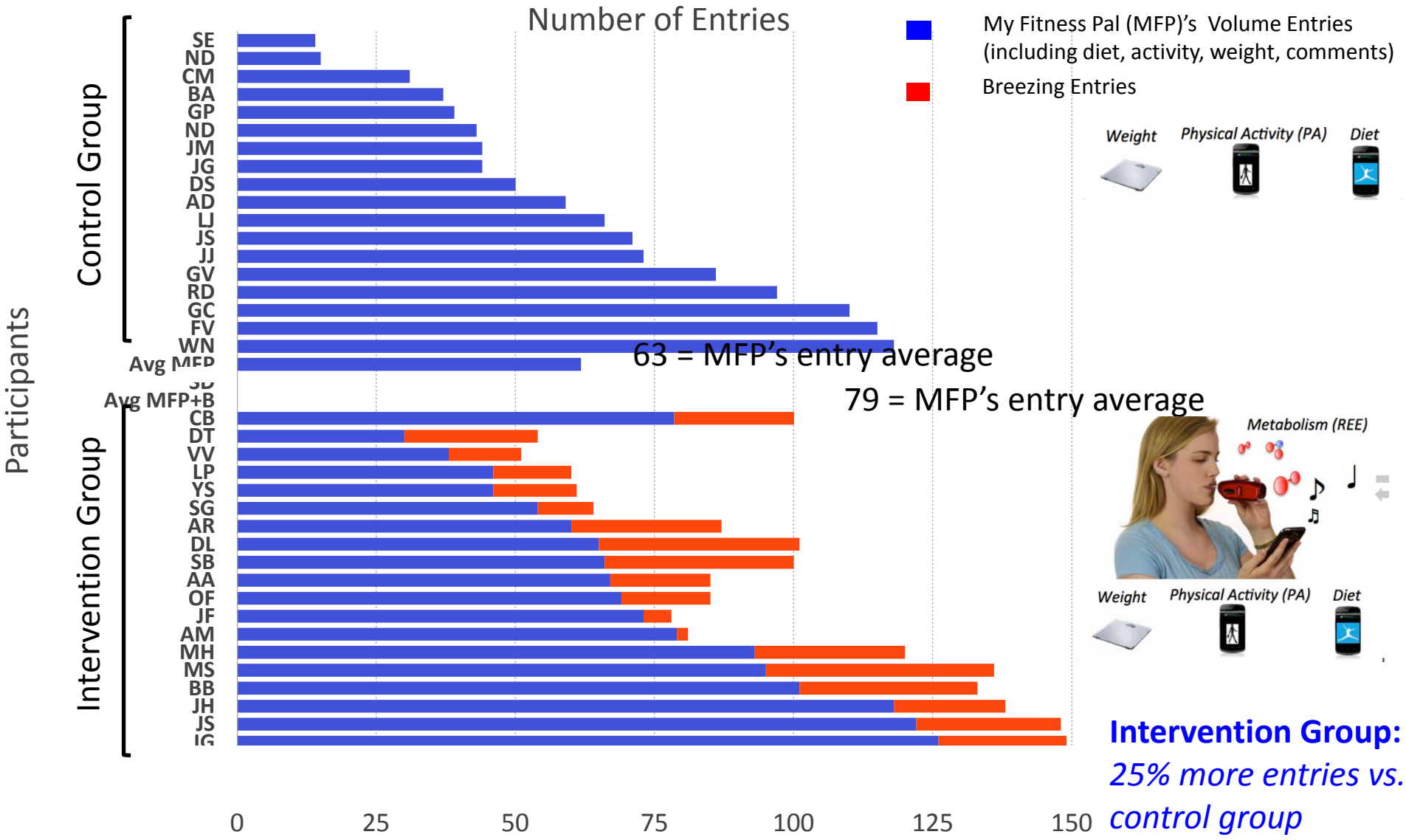
Case #2: Calorie Intake Complete Days*



The Intervention group had 70% more entries of completed daily calorie intake than the control group

* Completed days represent calorie intake values with equal or 25%+ of recommended calorie intake

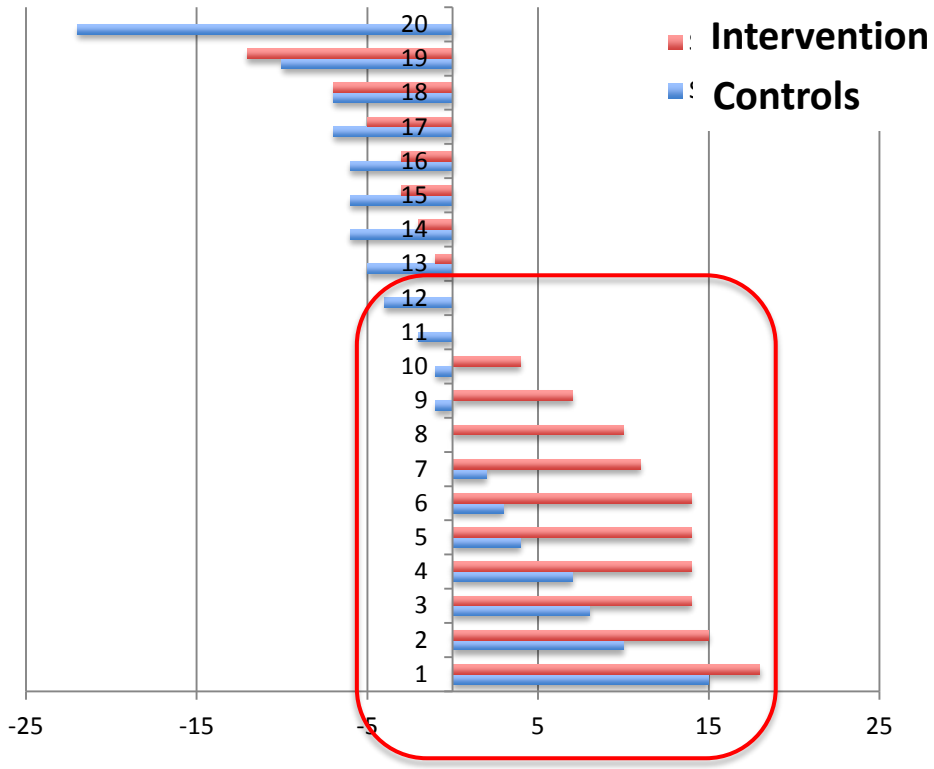
Case #2: Calorie Intake Entries



Intervention Group:
25% more entries vs. control group

Case #2: Benefits of weight loss in blood parameters

HDL change



Intervention group had a better outcome for HDL cholesterol (increased HDL cholesterol with a significant difference of p = 0.037 with respect to the control group

Diastolic Blood Pressure

Intervention group had a better outcome for reduction of diastolic blood pressure: a decrease with a significant difference of p = 0.07 with respect to the control group

Summary of facts from the study

1. **Breezing users had:**

i) Effectively lost more weight (89% vs 55% controls)

ii) Completed 70% more calorie intake inputs to Calorie Counter App

iii) More comprehensive use **of calorie counter app via entry volumes of diet, activity, weight, and comments.**

iv) Better HDL cholesterol and Diastolic Blood Pressure parameter outcomes

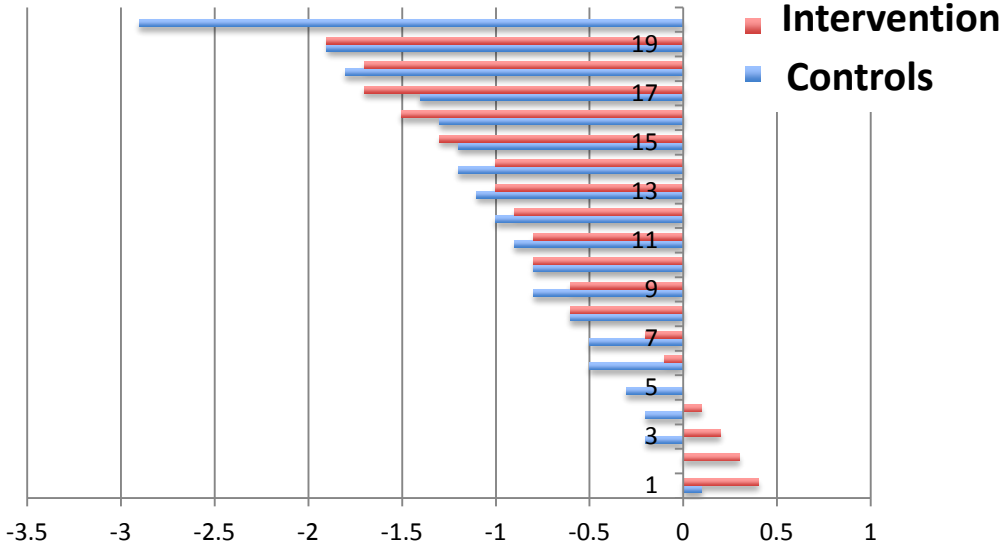
2. How does knowing Correct Calories Burned relate to Weight Loss?

89% efficiency of weight loss (IG) *vs.* **55%** efficiency of weight loss (CG)

5% of weight gain (IG) *vs.* **40%** of weight gain (CG)

General weight loss effect in T2 diabetes

HbA1c reduction



The weight reduction resulted in a reduction of glycated hemoglobin in both groups ($p < 0.1$)

Since both groups had a relatively high rate of weight loss (89%-IG and 55%-CG), there was not significant difference between groups in regard to improvements of glycated hemoglobin (both groups did improved the T2 diabetes parameter)

CONCLUSION: weight loss is a great intervention for decreasing T2 diabetes and risk of Diabetes

Controls

Unpaired *t* test results

P value and statistical significance:
The two-tailed P value equals 0.0438
By conventional criteria, this difference is considered to be statistically significant.

Confidence interval:
The mean of Group One minus Group Two equals 0.9300
95% confidence interval of this difference: From 0.0274 to 1.8326

Intermediate values used in calculations:
t = 2.0858
df = 38
standard error of difference = 0.446

Learn more:
GraphPad's web site includes portions of the manual for GraphPad Prism that can help you learn statistics. First, review the meaning of P values and confidence intervals. Then learn how to interpret results from an unpaired or paired *t* test. These links include GraphPad's popular *analysis checklists*.

Review your data:

Group	Group One	Group Two
Mean	6.8100	5.8800
SD	1.4100	1.4100
SEM	0.3153	0.3153
N	20	20

Intervention

Unpaired *t* test results

P value and statistical significance:
The two-tailed P value equals 0.1070
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Group One minus Group Two equals 0.6900
95% confidence interval of this difference: From -0.1566 to 1.5366

Intermediate values used in calculations:
t = 1.6530
df = 36
standard error of difference = 0.417

Learn more:
GraphPad's web site includes portions of the manual for GraphPad Prism that can help you learn statistics. First, review the meaning of P values and confidence intervals. Then learn how to interpret results from an unpaired or paired *t* test. These links include GraphPad's popular *analysis checklists*.

Review your data:

Group	Group One	Group Two
Mean	6.6100	5.9200
SD	1.4100	1.1500
SEM	0.3235	0.2638
N	19	19

Between groups: no difference

Unpaired *t* test results

P value and statistical significance:
The two-tailed P value equals 0.2462
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Group One minus Group Two equals -0.2736843000
95% confidence interval of this difference: From -0.7442892210 to 0.1969206210

Intermediate values used in calculations:
t = 1.1783
df = 37
standard error of difference = 0.232

Learn more:
GraphPad's web site includes portions of the manual for GraphPad Prism that can help you learn statistics. First, review the meaning of P values and confidence intervals. Then learn how to interpret results from an unpaired or paired *t* test. These links include GraphPad's popular *analysis checklists*.

Review your data:

Group	Group One	Group Two
Mean	-0.9631580000	-0.6894737000
SD	0.7251130000	0.7248714300
SEM	0.1621401959	0.1662969111
N	20	19

THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE

ARIZONA STATE UNIVERSITY

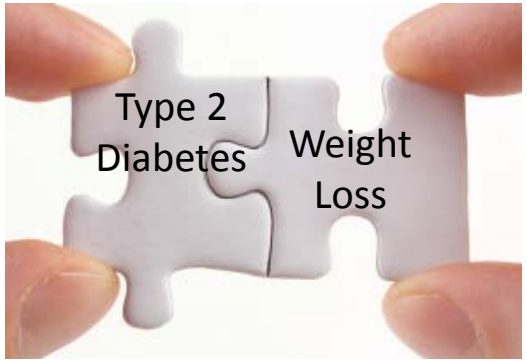
BRIDGING THE GAP

Research World

Data Analysis

Real World

Medical and tracking devices



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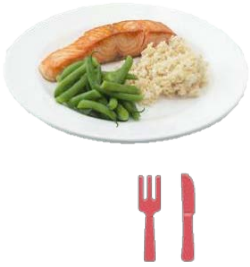

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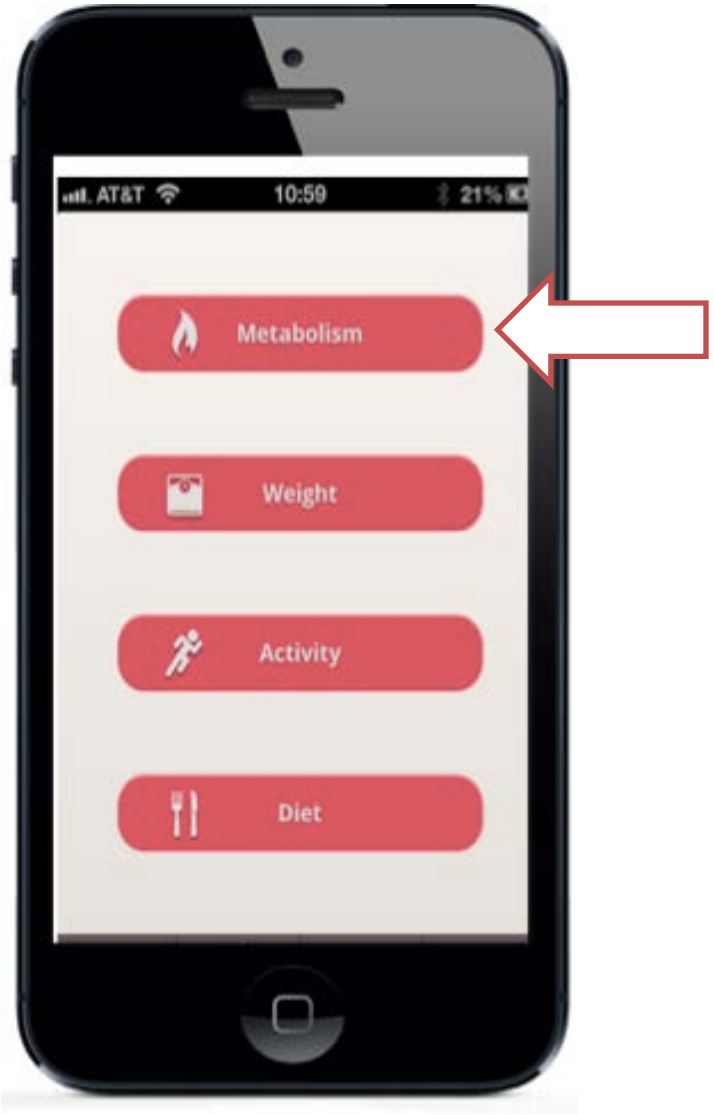
Demo of Metabolism Tracking



- Miscellaneous slides

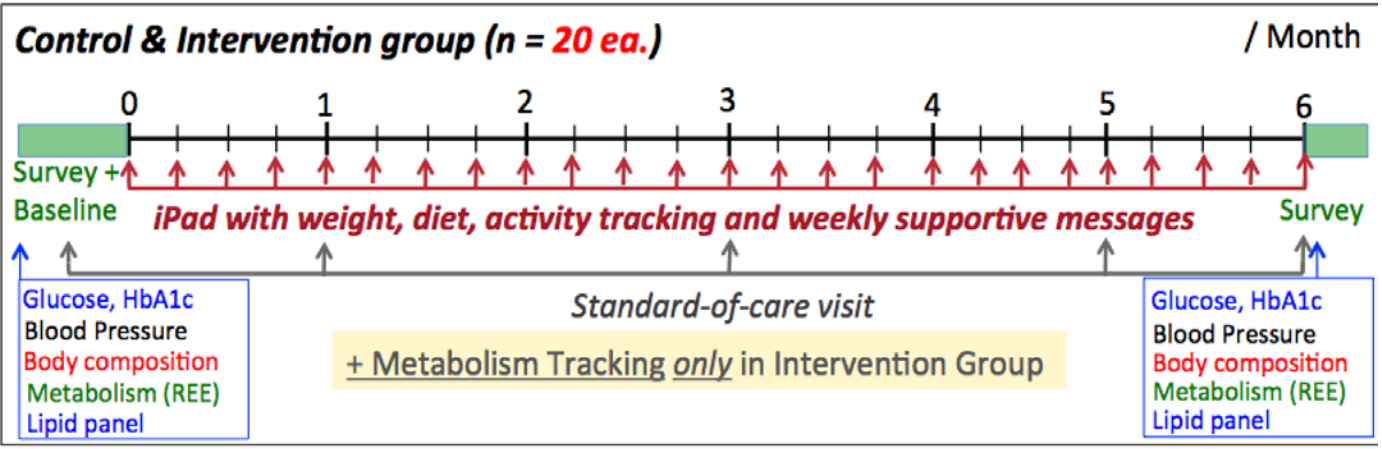
Six-month study design

Control Group (CG)

Intervention Group (IG)

•The participants from the control group had an iPad with My Fitness Pal App to track calorie intake, an activity tracker to track steps and floors, and a weight scale.

• Each participant in the control group was recommended a 500-calorie deficit intake based on the Harris Benedict Equation



Baseline: initial measurement period; **Surveys:** subjects pre- and post study survey, Metabolism tracker: Breezing

• The intervention group had the same gadgets as the control group, as well as a Breezing Tracker.

• Both groups were followed up with a Standard-of-Care procedure for 6 months, and were reached by e-mail every 2-3 weeks with general health information.

** Most of participants had T2 Diabetes, or were at risk of Diabetes*

A First Law of Thermodynamics: Energy Conservation Law

Weight = *Caloric Intake* - *Total energy expenditure (TEE)*

Resting Metabolic Rate (RMR)

80-90 %

(sedentary)

+

Physical Activity

< 20 % (sedentary)

Affordable & Mobile Technologies

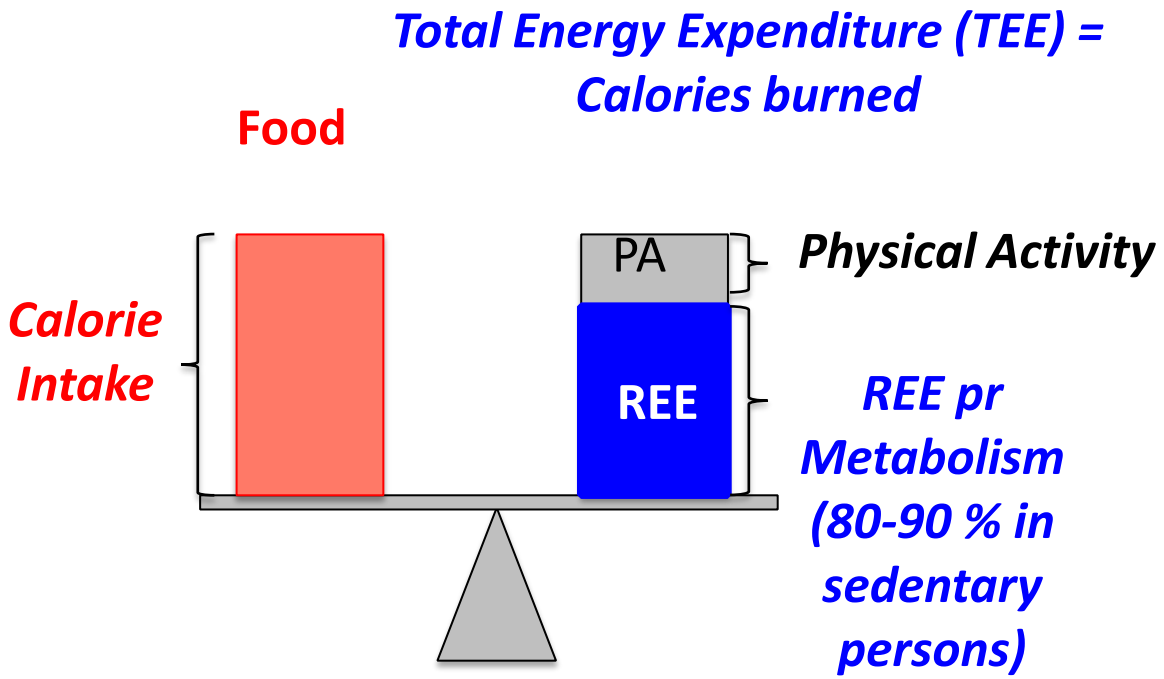
Breezing

Metabolic Rate Tracker

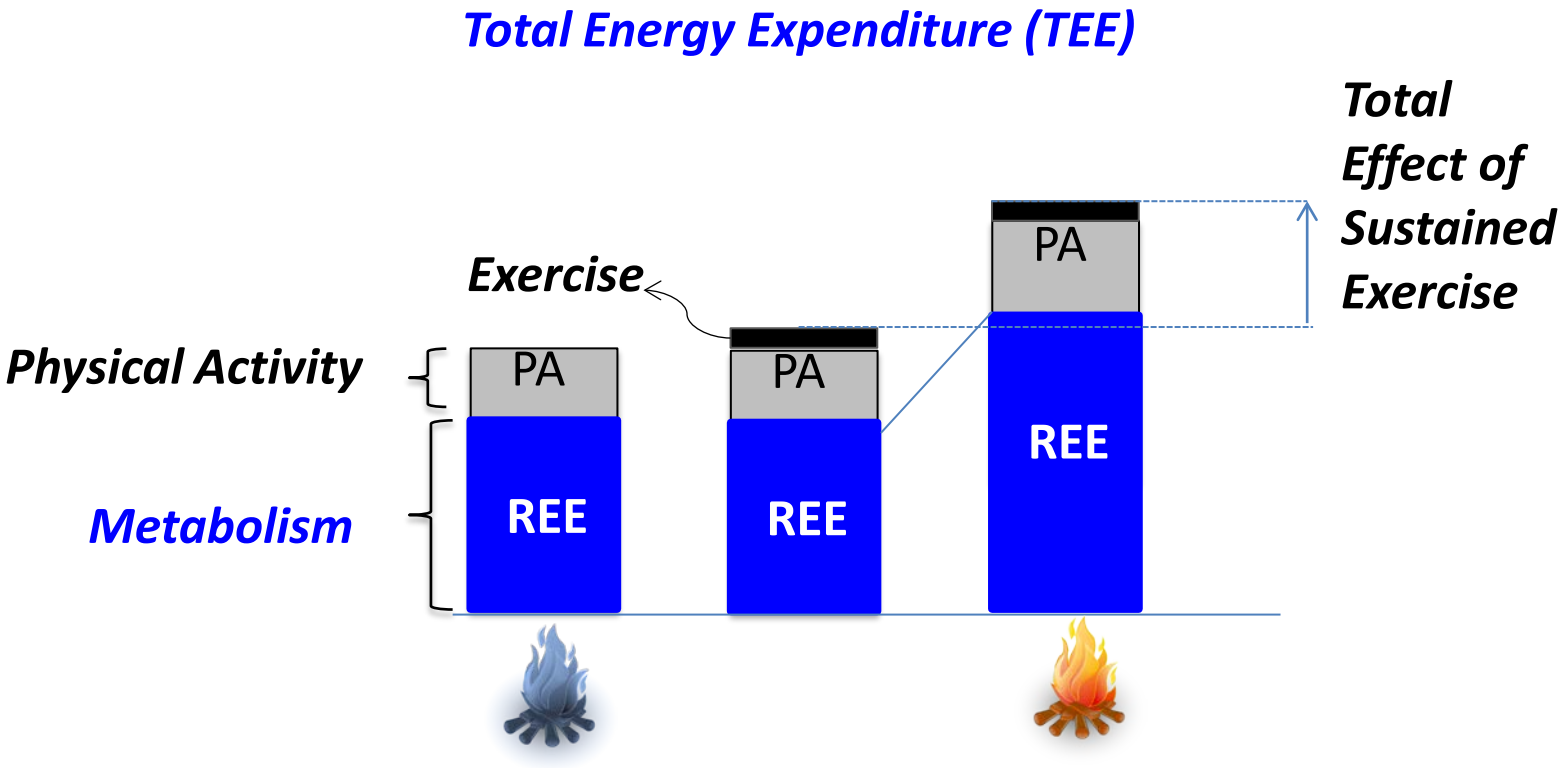
Affordable & Mobile Technologies

What about the variability of REE?

Energy Balance – How we can modify it?



Metabolism (RMR) and Physical Activity*

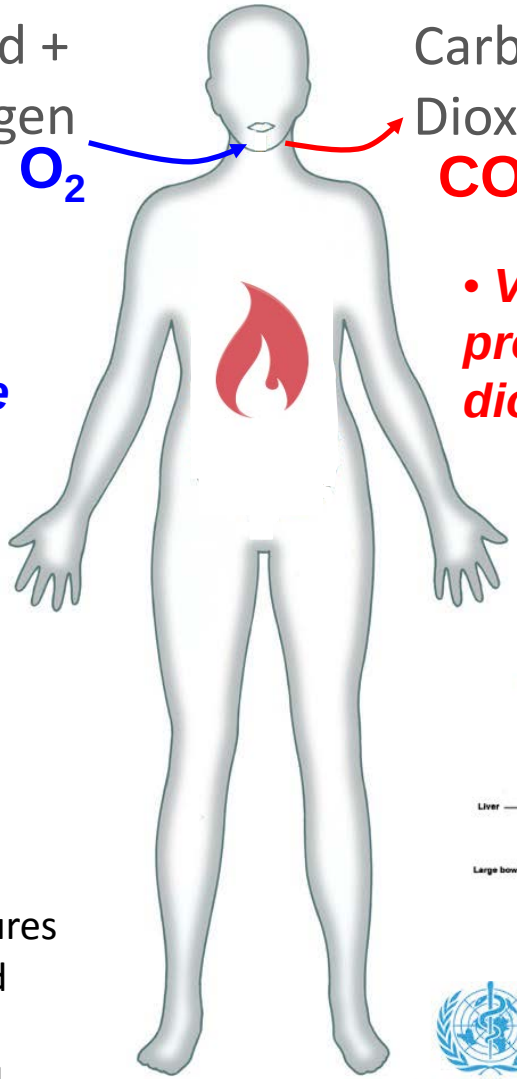


*Speakman et.al., Proceeding of the nutrition society, 2003, 62, 621-634 (Fig.2 reproduction)

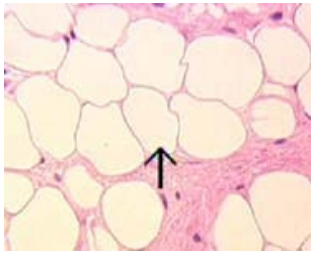
Resting Energy Expenditure (REE)
Resting Metabolic Rate (RMR)

Food + Oxygen → Carbon Dioxide + ATP (heat), storage

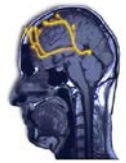
O_2 CO_2

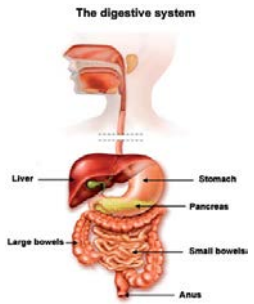


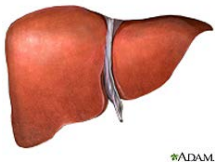
- ***VO_2 consumed oxygen rate***
- ***VCO_2 produced carbon dioxide rate***

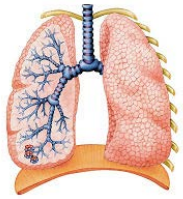


Resting Metabolism:
- ✓ *Sustains life*
- ✓ *Majority of our daily total energy expenditure*









How does it work?

- Indirect calorimetry (Breezing measures consumed oxygen rate and produced carbon dioxide rate)
- Recommended by AND, WHO, ACSM, ADA

World Health Organization

American College of Sports Medicine, Founded 1954

American Diabetes Association®
Cure • Care • Commitment®

eat right® Academy of Nutrition and Dietetics

Resting Energy Expenditure:

Indirect Calorimetry Principle

Weir Equation:

$$REE\ (kCal/day) = [3.9\ (VO_2) + 1.1\ (VCO_2)] \times 1.44$$

VO_2: consumed oxygen rate (mL/min)
VCO_2: produced carbon dioxide rate (mL/min)

Weir, J. B. D. (**1949**). "New Methods For Calculating Metabolic Rate With Special Reference To Protein Metabolism." Journal Of Physiology-London **109**(1-2): 1-9.

Weir, J. B. D. (**1990**). "Nutrition Metabolism Classic - New Methods For Calculating Metabolic-Rate With Special Reference To Protein-Metabolism." Nutrition **6**(3): 213-221.

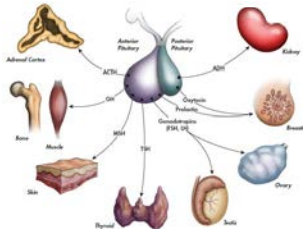
Why track metabolism?

Different factors can affect metabolism

Genetics

Exercise

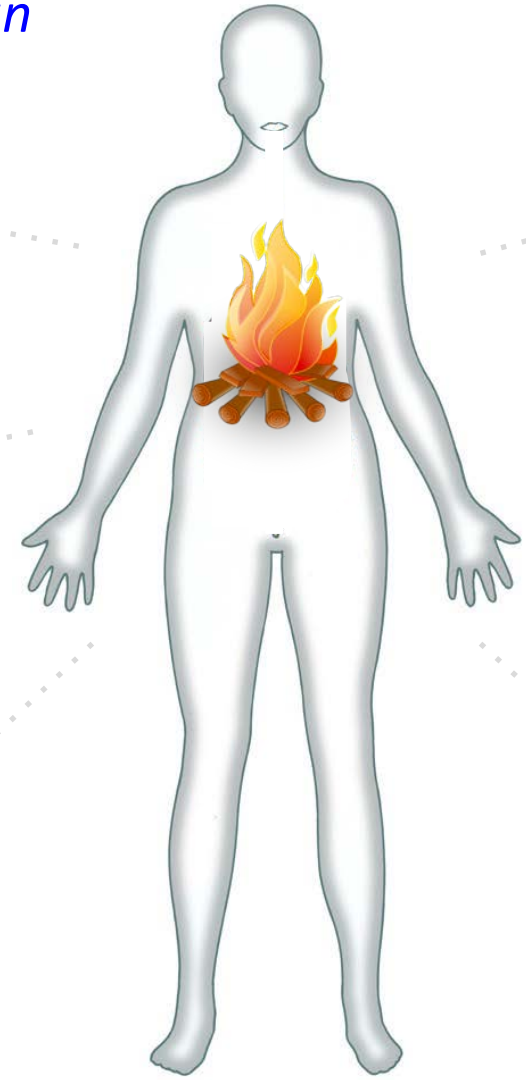
Hormones



Drugs, substances

Diet

Pregnancy

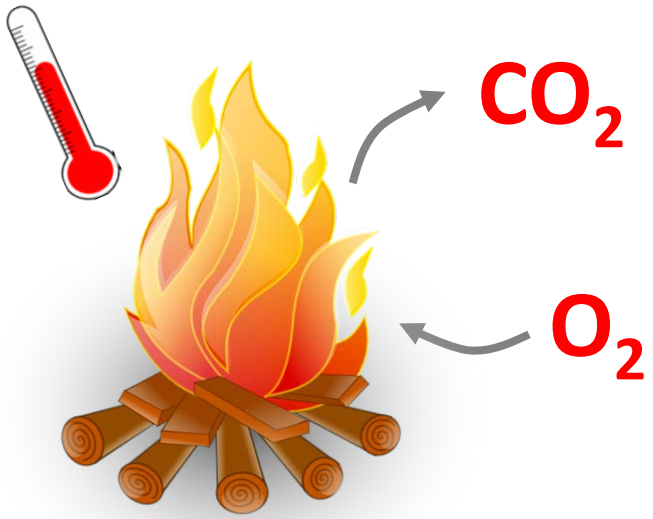


Informacion from Sport Nutrition for Health and Performance, M. Manore, N. Mayer, J. Thompson - 2009

Direct Calorimetry vs. Indirect Calorimetry

Direct Heat Measurement

Indirect Heat Measurement

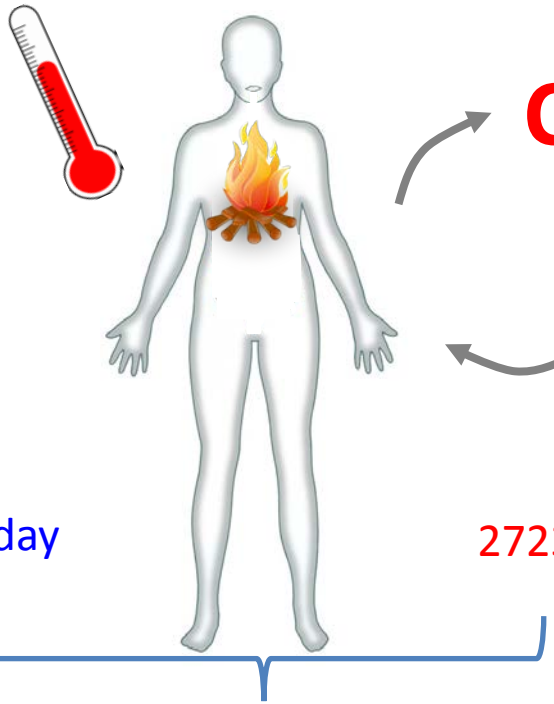


Direct Calorimetry vs. Indirect Calorimetry

Direct Heat Measurement

Indirect Heat Measurement

1900 - Atwater & Rosa' research:
Energy expenditure of 3 men who lived in the calorimeter for 40 days



2717 kCal/day

2723 kCal/day

Error: +/- 0.2 %

* Other researchers' experiments: Error = +/- 1%

Science demonstrated that direct calorimetry is equivalent to indirect calorimetry

History of Measuring Energy Expenditure

1770's

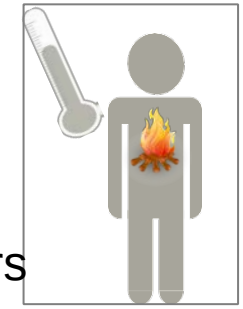
Lavoisier & Laplace

Law of the conservation of energy → First Human Calorimetry

1890's

Atwater & Rosa

Wesleyan Univ.: First important work of Direct Calorimetry



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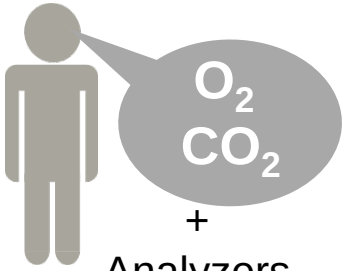
Analyzers

1900's

Other Direct Calorimetry Efforts:

Airflow, water flow, water storage, and gradient layer calorimeter

Claude Douglas: Indirect Calorimetry Gold Standard Method



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Analyzers

1940

Indirect Calorimetry: Open circuit spirometry

12 lbs

1980

Indirect calorimetry: Computer-based Instrumentation (**Breath-by-breath**)

2000

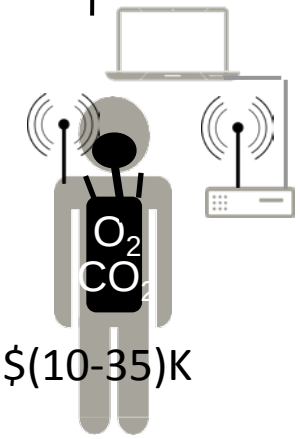
Portable Breath-by-Breath Instrumentation

2010

2014

First Mobile Metabolism Tracker

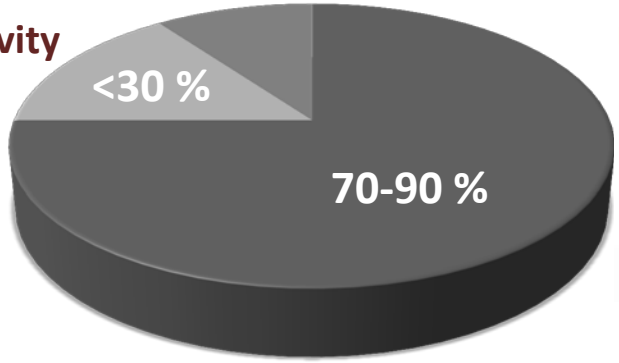
$350



$(10-35)K

Total Energy Expenditure*

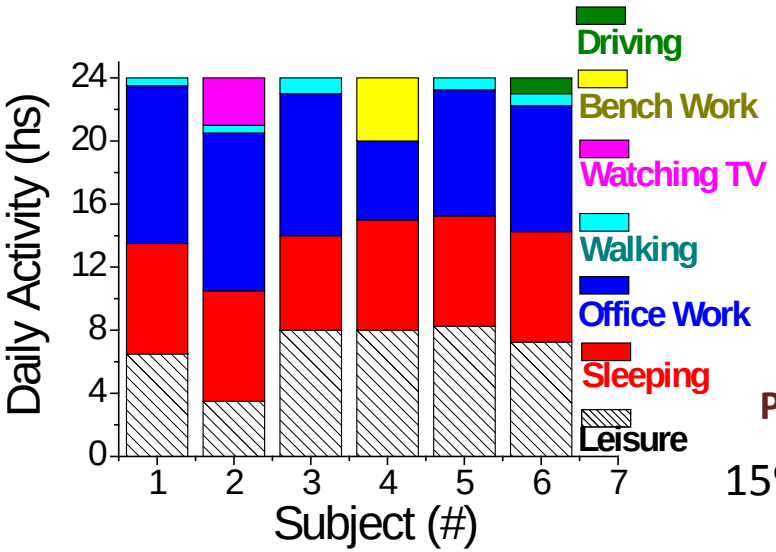
Physical Activity



Resting Metabolic rate or
Resting Energy Expenditure

*McArdle, Katch & Katch, Ex. Physiology, 2009

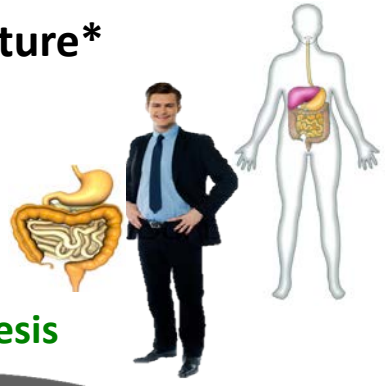
How Sedentary Are We?



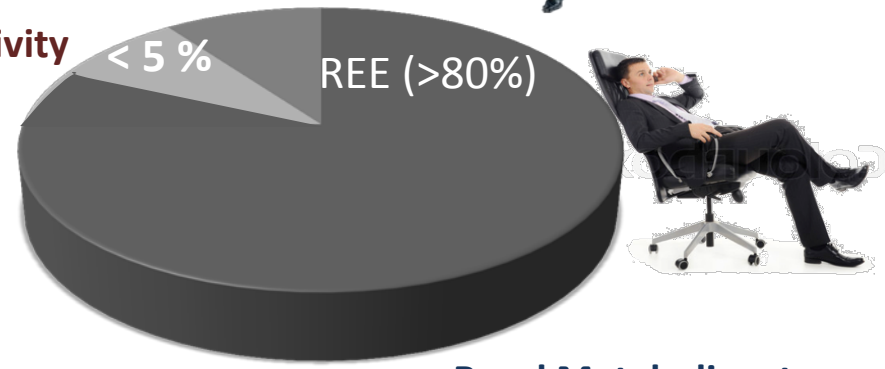
Physical Activity

15% → 5%

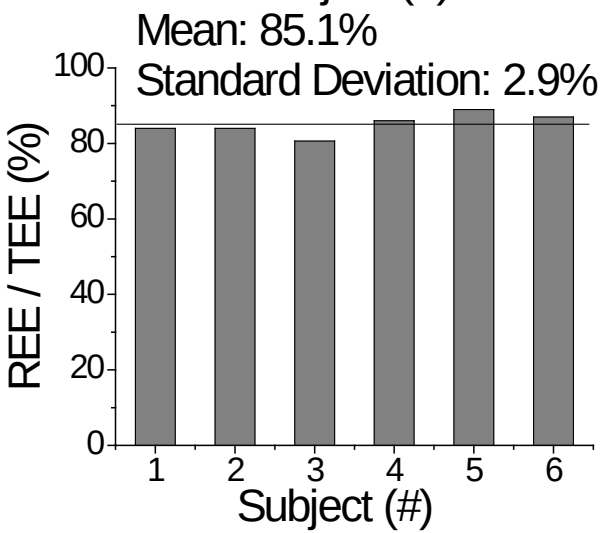
Total Energy Expenditure*



Thermogenesis



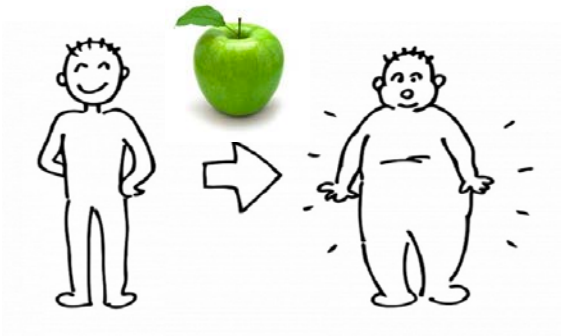
Basal Metabolic rate



Most of daily total energy expenditure (TEE) is spent to maintain basic body functions (energy expenditure at resting state, REE)

Work from Arizona State University, 2013

The risk of using calorie intake recommendations from an equation-based REE value



BUSINESS

Share:

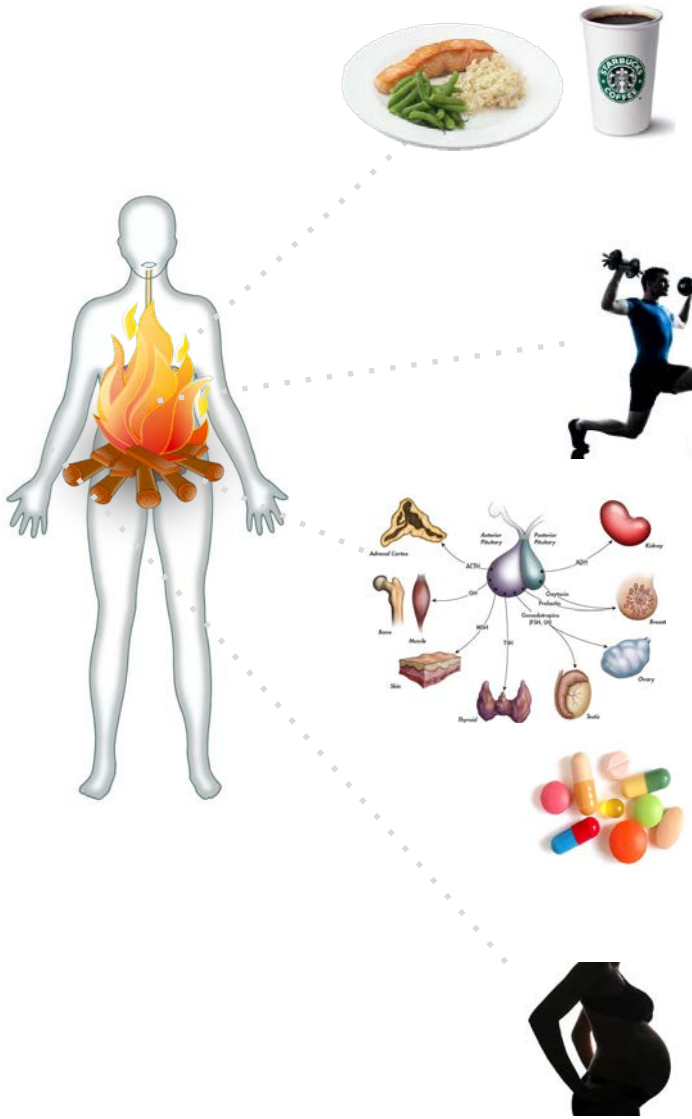
Weekly Wellness: The sad state of the American metabolism

AP Photo/Paul Vernon

Matt Gallagher | MFC Sports Performance | Submitted
June 9 2 p.m.

Our weekly fitness column, "Weekly Wellness," is back again. This week, Matt Gallagher, from MFC Sports Performance in Darien, discusses why Americans weight issues have a lot to do with our poor metabolism.

Tracking Metabolism for Better Health



Diet

Changes in diet can significantly change metabolism. For example, a crash diet can cause drastic reduction in metabolic rate, leading to a “weight loss plateau”. See slides in next class.

Exercise

Exercise can affect metabolism. For example, muscle-building increases metabolism and High Intensity Intermittent Training (HIIT) creates an “afterburn” effect. See slides in next class.

Hormones/medication

Hormonal changes and medications can change metabolism. Monitoring metabolism helps screen for potential hyper- or hypo-thyroidism. See slides in next class.

Pregnancy

Metabolism changes significantly throughout pregnancy and after giving birth. Tracking metabolism helps the mother maintain and achieve the proper weight for the baby’s healthy growth. See slides in next class.

Tracker for Resting Energy Expenditure (REE) or Resting Metabolic Rate

Global Journal of Obesity, Diabetes and Metabolic Syndrome

Peertechz

Xiaojun Xian, Ashley Quach, Devon Bridgeman, Francis Tsow*, Erica Forzani* and Nongjian Tao*

Center for Bioelectronics & Biosensors, the Biodesign Institute, Arizona State University, Tempe, Arizona 85287, USA

Dates: Received: 30 October, 2014; **Accepted:** 19 March, 2015; **Published:** 21 March, 2015

***Corresponding authors:** Francis Tsow, Center for Bioelectronics & Biosensors, the Biodesign Institute, Arizona State University, Tempe, Arizona 85287, USA, E-mail: tsing.tsow@asu.edu

Erica Forzani, Center for Bioelectronics & Biosensors, the Biodesign Institute, Arizona State University, Tempe, Arizona 85287, USA, E-mail: erica.forzani@asu.edu

Research Article

Personalized Indirect Calorimeterfor Energy Expenditure (EE) Measurement

Abstract

Background and aims: A personal indirect calorimeter allows everyone to assess resting and non-resting energy expenditure, thus enabling accurate determination of a person's total calorie need for weight management and fitness. The aim of this study is to compare the performance of a new personal metabolic rate tracker based on indirect calorimetry, Breezing®, with the Douglas bag method, the gold standard method for energy expenditure (EE) measurement.

Methods: Energy expenditures (EE) at rest and during activities, and respiratory quotient (RQ) were measured for 12 healthy subjects, including 7 males and 5 females under different living conditions. A total of 314 measurements were performed with Breezing®, and the results were compared with those by the Douglas bag method.

Results: R-squared correlation coefficients (R^2) between the data obtained with Breezing® and the Douglas bag method were 0.9976, 0.9986, 0.9981, and 0.9980, for VO_2, VCO_2, EE, and RQ respectively.

Conclusions: The EE and RQ values determined by Breezing® are in good agreement with those by the Douglas bag method.

GJODMS, March, 2015

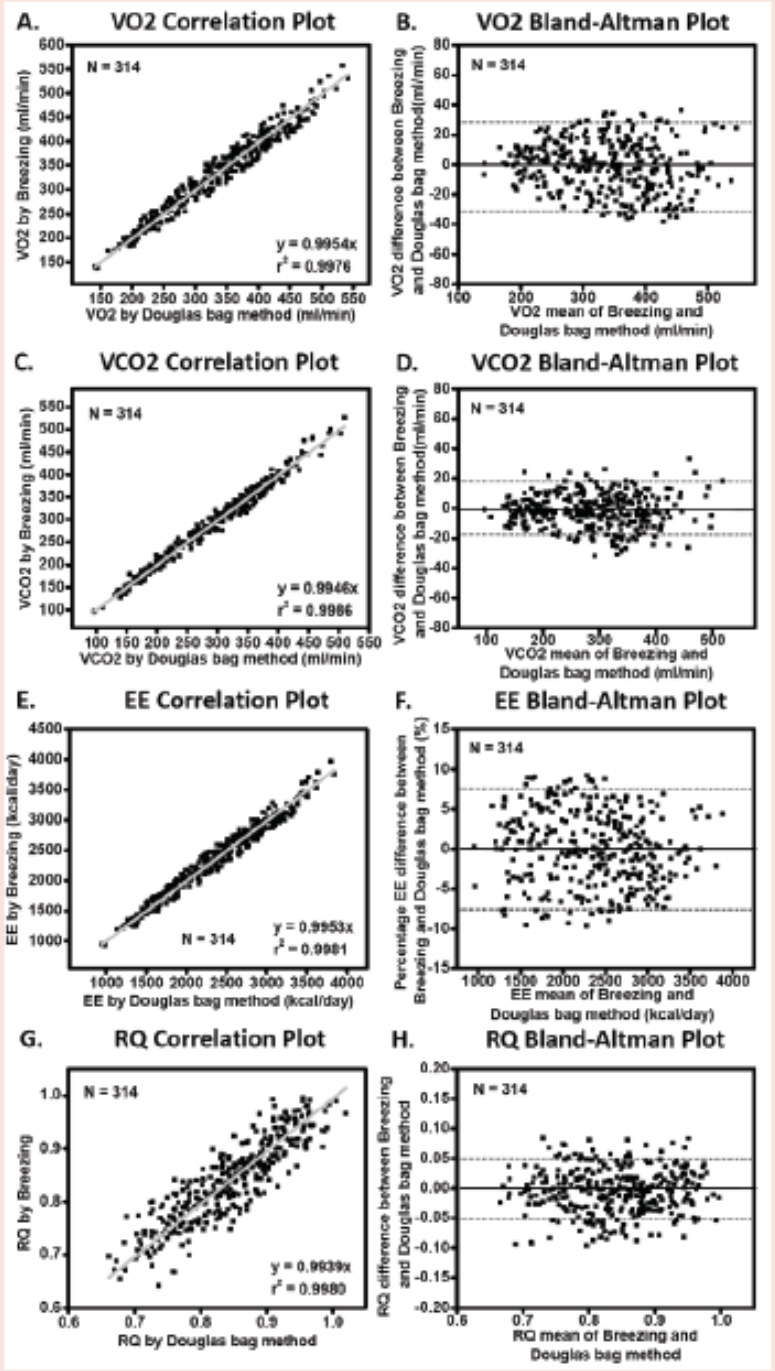


Figure 2: Comparison between the Breezing® device and Douglas bag method. (A) VO_2 correlation plot; (B) VO_2 Bland-Altman plot; (C) VCO_2 correlation plot; (D) VCO_2 Bland- Altman plot; (E) EE correlation plot; (F) EE Bland-Altman plot (in percentage); (G) RQ correlation plot; (H) RQ Bland-Altman plot.

Breezing

The Tracker for Energy Expenditure (EE) demonstrated ~100% accuracy

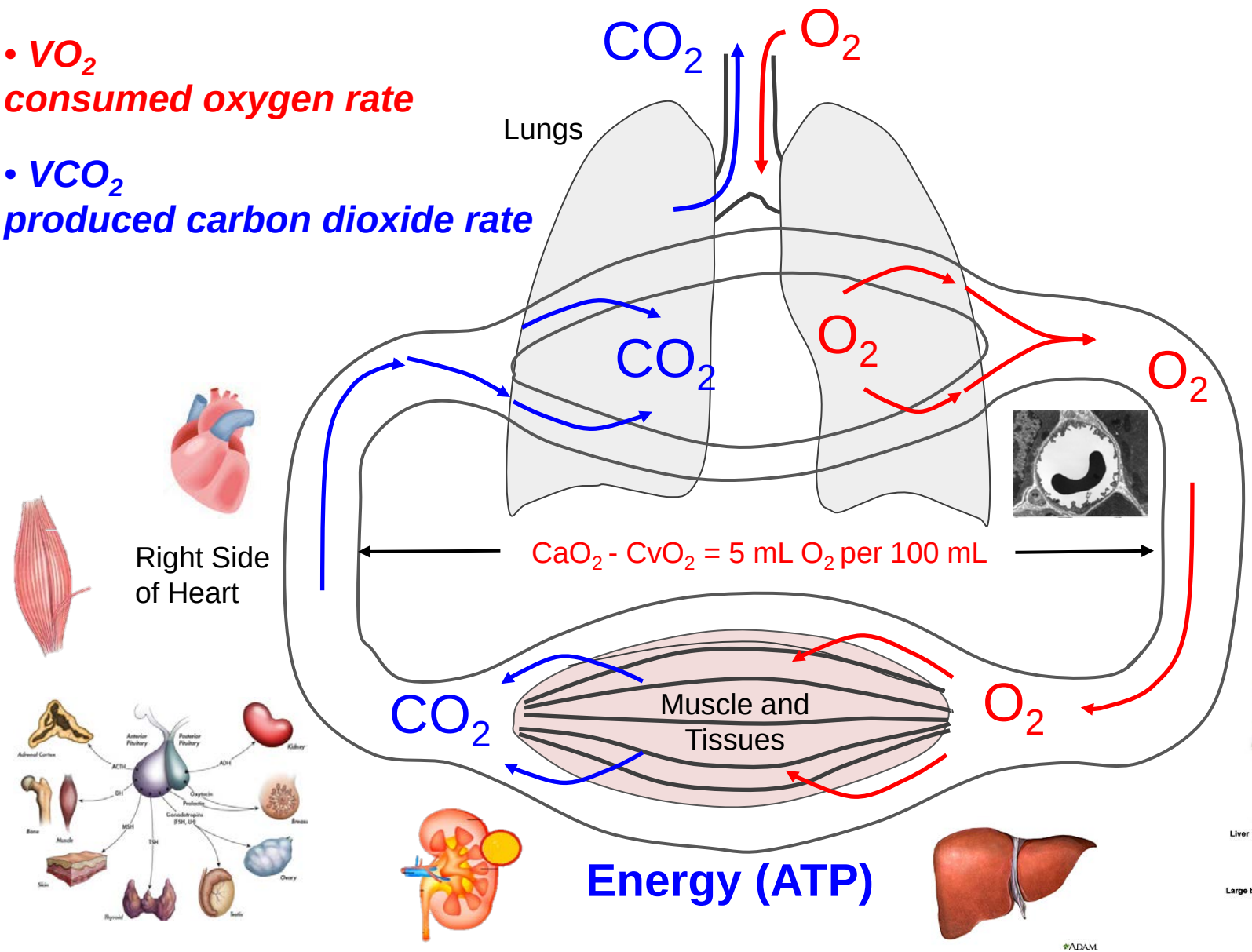
GJODMS, March, 2015

http://www.peertechz.com/Obesity-Diabetes-Metabolic-Syndrome/GJODMS-2-107.php

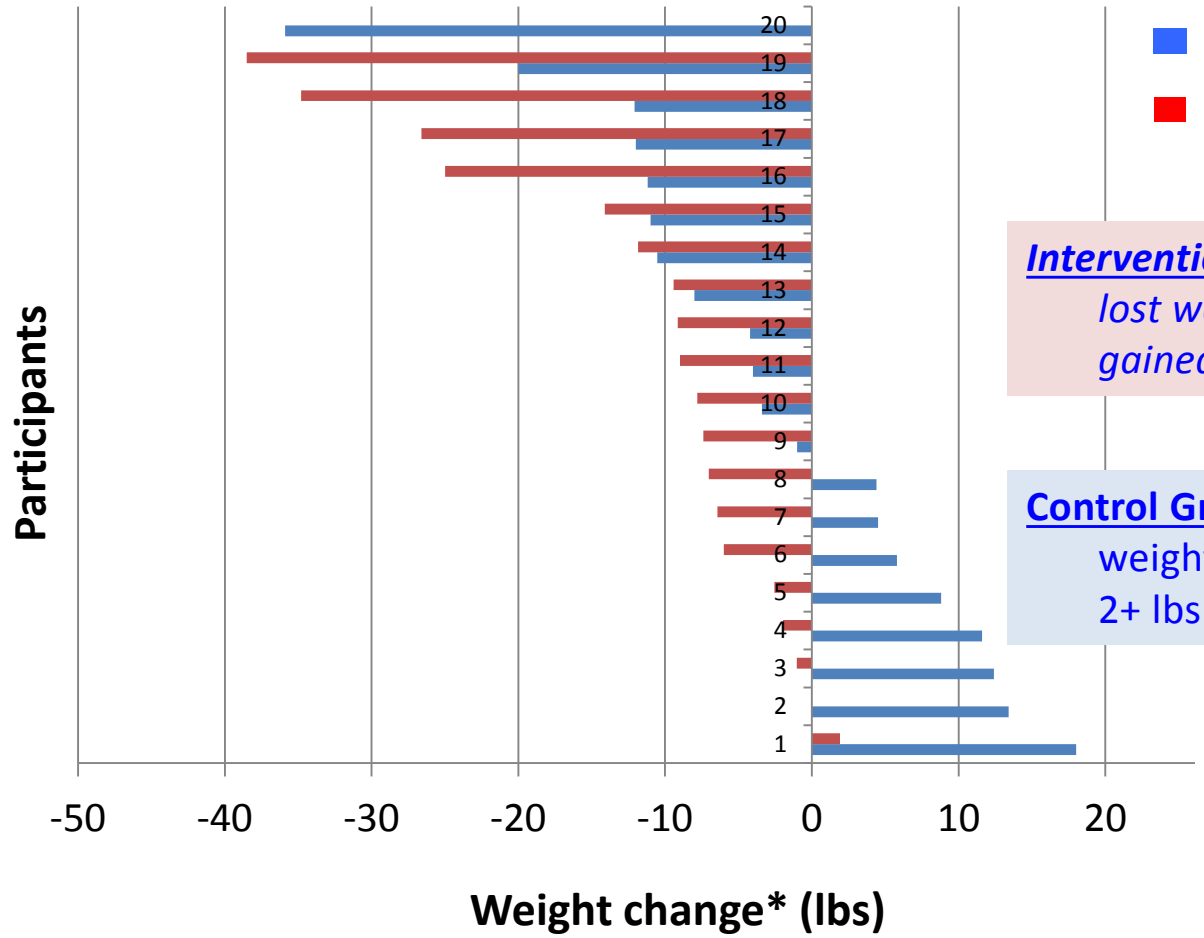
Energy management: Cardio-Pulmonary System

- ***VO_2***
consumed oxygen rate

- ***VCO_2***
produced carbon dioxide rate



Case #2: Weight & Body Mass changes



Control Group (CG)

Intervention Group (IG)

Intervention Group: *17 of 19 participants (**89%**) lost weight, 1 stayed steady and 1 (5%) gained 1.9 lbs.*

Control Group: 11 of 20 participants (**55%**) lost weight, 1 stayed steady and 8 (**40%**) gained 2+ lbs.

Other results:

Weight loss Greater Than 6 lbs:
*CG: **40%** (8/20) **vs** IG: **68%** (13/19)*

Observation: Weight change is accounted from 1st day the participant use MFP (baseline period) up to 6 months after the study

HONORHEALTH™

Ventilator Associated Pneumonia

"Genomics and Personalized Medicine"
HonorHealth Research Institute

Charles Hu, MD
Emmanuel Menashi, PhD

Ventilator Associated Pneumonia (VAP)

- Healthcare Associate Infection (HAI)
- High anti-biotic use
- Morbidity/Hospitalization/Cost
- Mortality

Clinical Diagnosis:

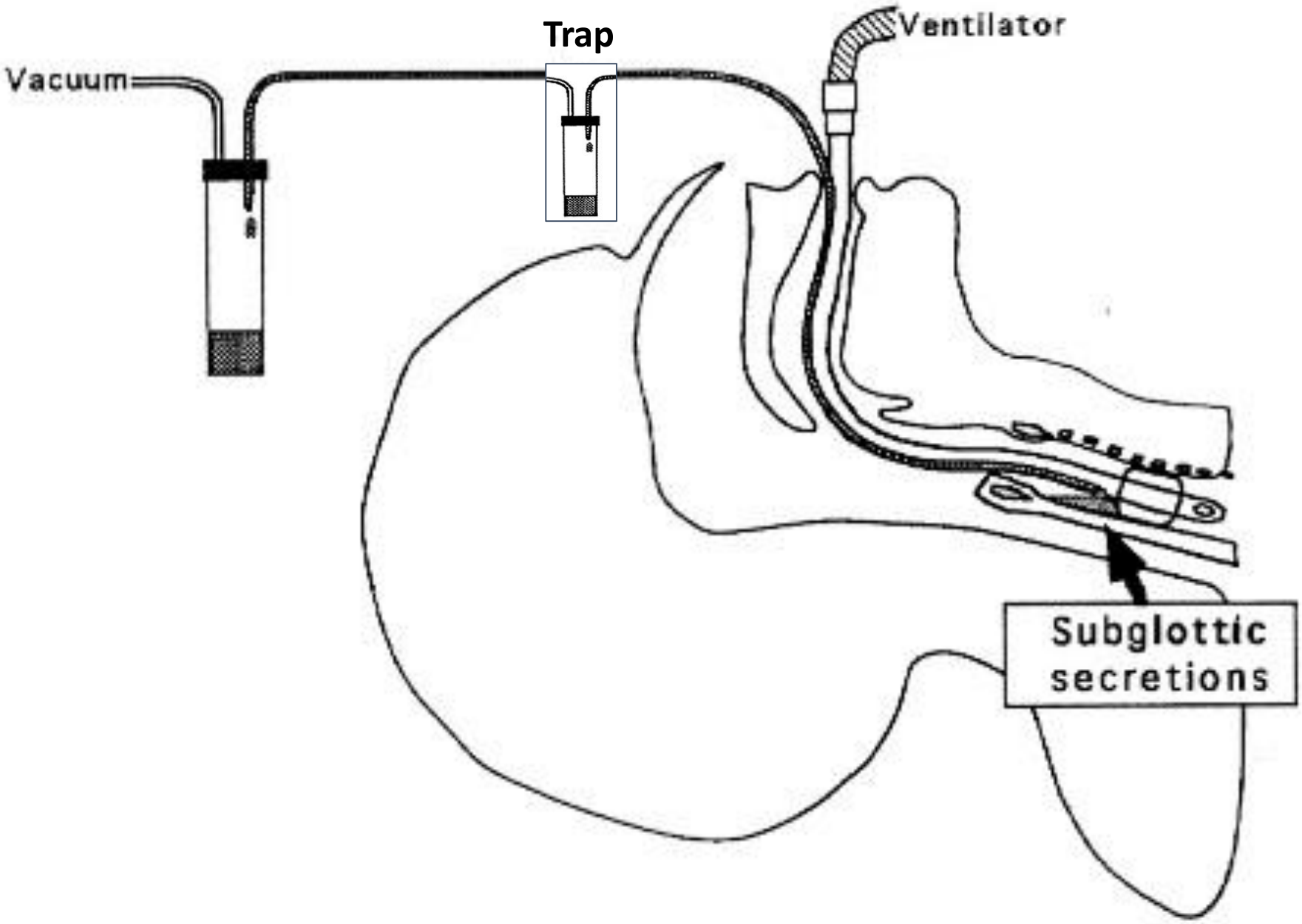
- **CPIS (Clinical Pulmonary Infection Score) System**

Clinical Pulmonary Infection Score (CPIS):

Parameter	Scores	
	1 point	2 points
Temp (°C)	38.5 to 38.9	≥39 or ≤36
White blood cells/mm^3	<4,000 or >11,000	<4,000 or >11,000 and ≥50% bands
Secretions Endo-Tracheal Aspirates (ETA)	Nonpurulent	Purulent
PaO_2/FiO_2		≤240 and no ARDS
Chest X-ray infiltrates	Diffuse or patchy	Localized

Detection

diagnosis


Trap
Ventilator
Vacuum
Subglottic
secretions

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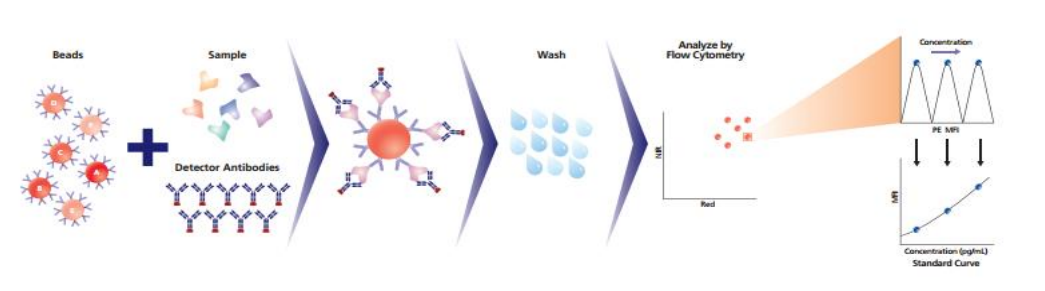
Methodologies:

1. Early Detection: Host's Immune Mediators

- **Flow-Cytometry:** Immune Mediators



- **HPLC/Mass-Spectroscopy**

  Shotgun Proteomic Approach, LC-MS/MS Peptide Analysis
  LTQ-Orbitrap Velos Mass Spectrometer

2. Diagnostic: Pathogenic Classification

Next Generation Sequencing: 16s rRNA (V1-V2)

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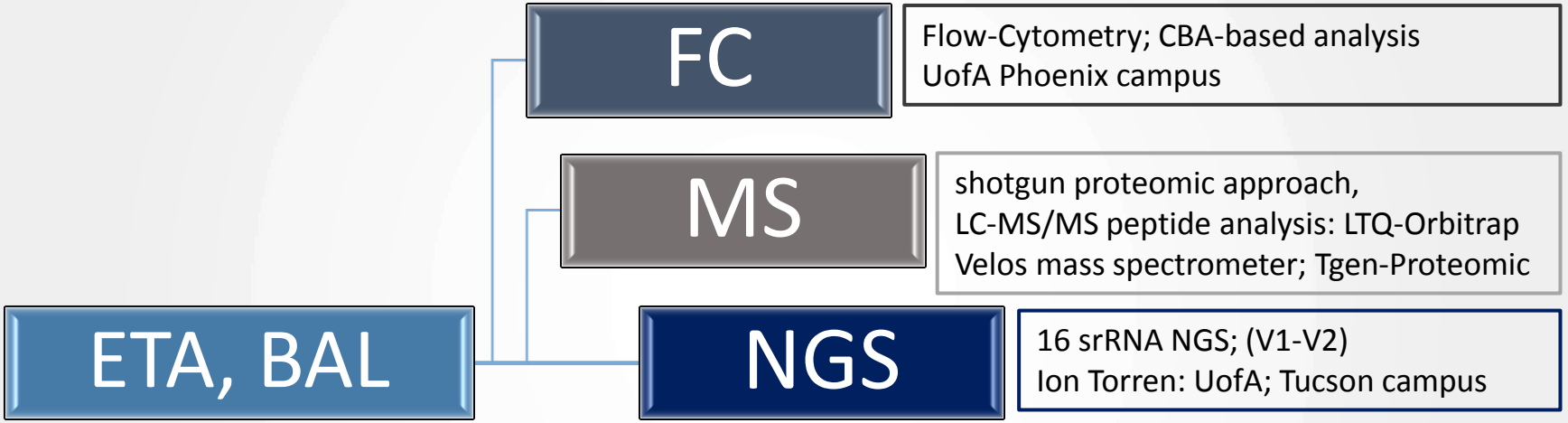
Biospecimens processing:



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Preliminary Data:

- **Subject-1:** **ETA-1, ETA-3, ETA-5, ETA-7 and ETA-9:** ***-No pneumonia***
- **Subject-2:** **ETA-1, ETA-3 and BAL** ***-Pneumonia (Aggressive)***
- **Subject-3:** **ETA-1, ETA-3, ETA-5 and BAL** ***-Pneumonia (Slow)***

Early Detection:

a- Complement System: (Anapylatoxin)

C3a, C4a and C5a

Biospecimens :

1. **Tracheal Aspirate**
2. **Bronchoalveolar Lavage BAL**

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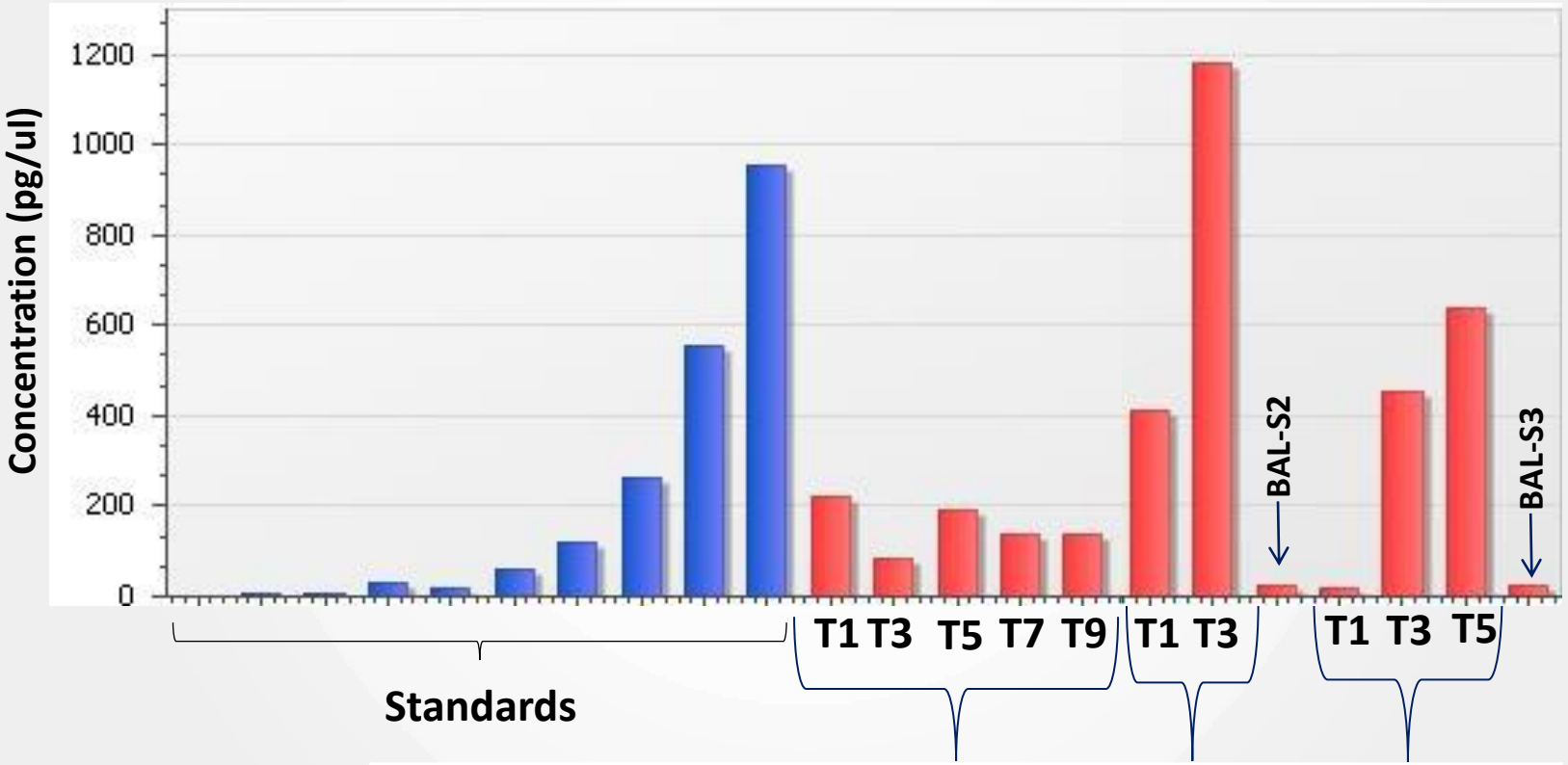
Anaphylatoxin C5a concentrations in ETA and BAL biospecimens



Subject	S1	S2	S3
Dilution factor	1/100	1/100	1/100

Early Detection:

b- Pro- and Anti- Inflammatory Immune Responses: (Th1/Th2/Th17)

- Screen seven Cytokines;

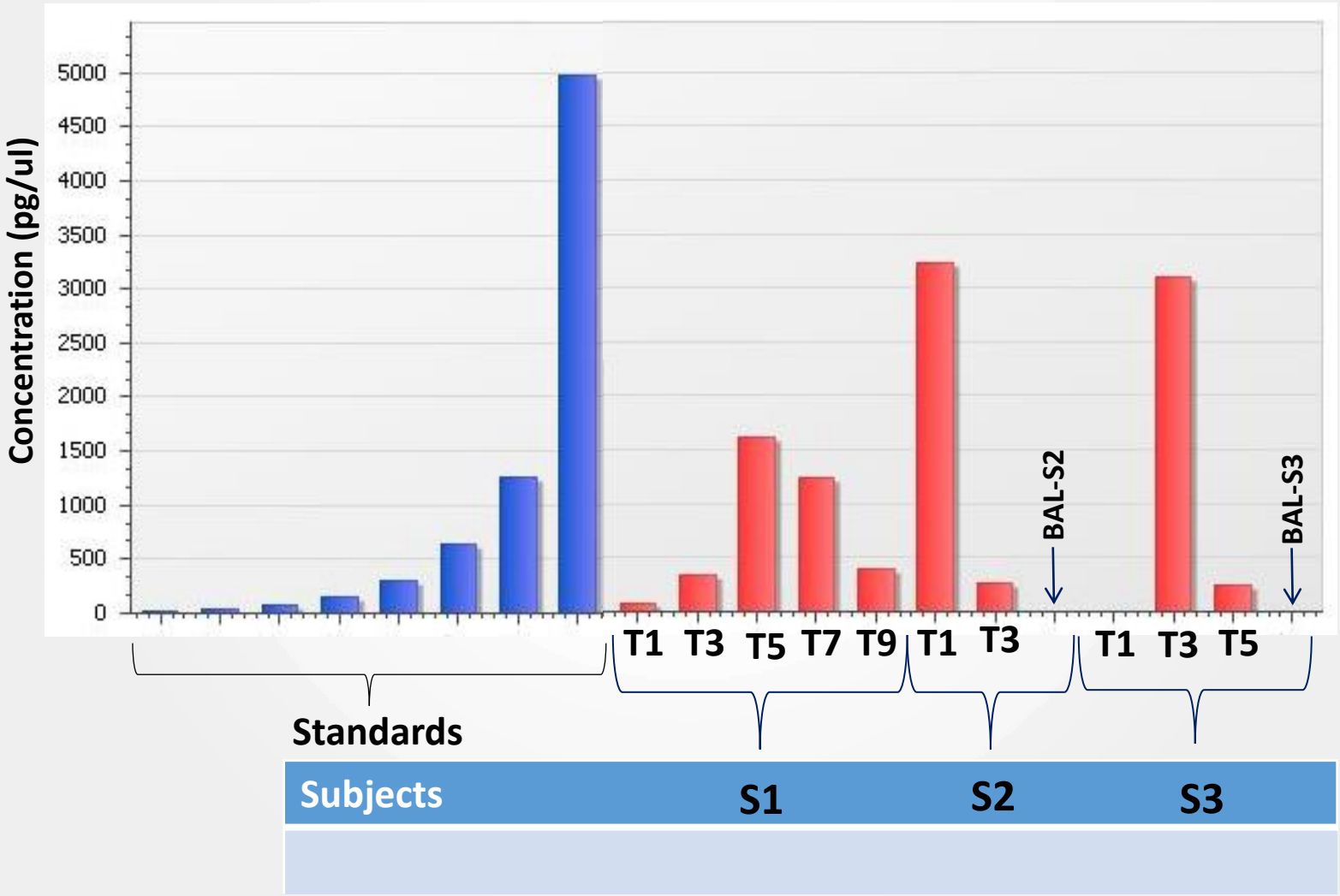
1. TNF-α
2. IL-6,

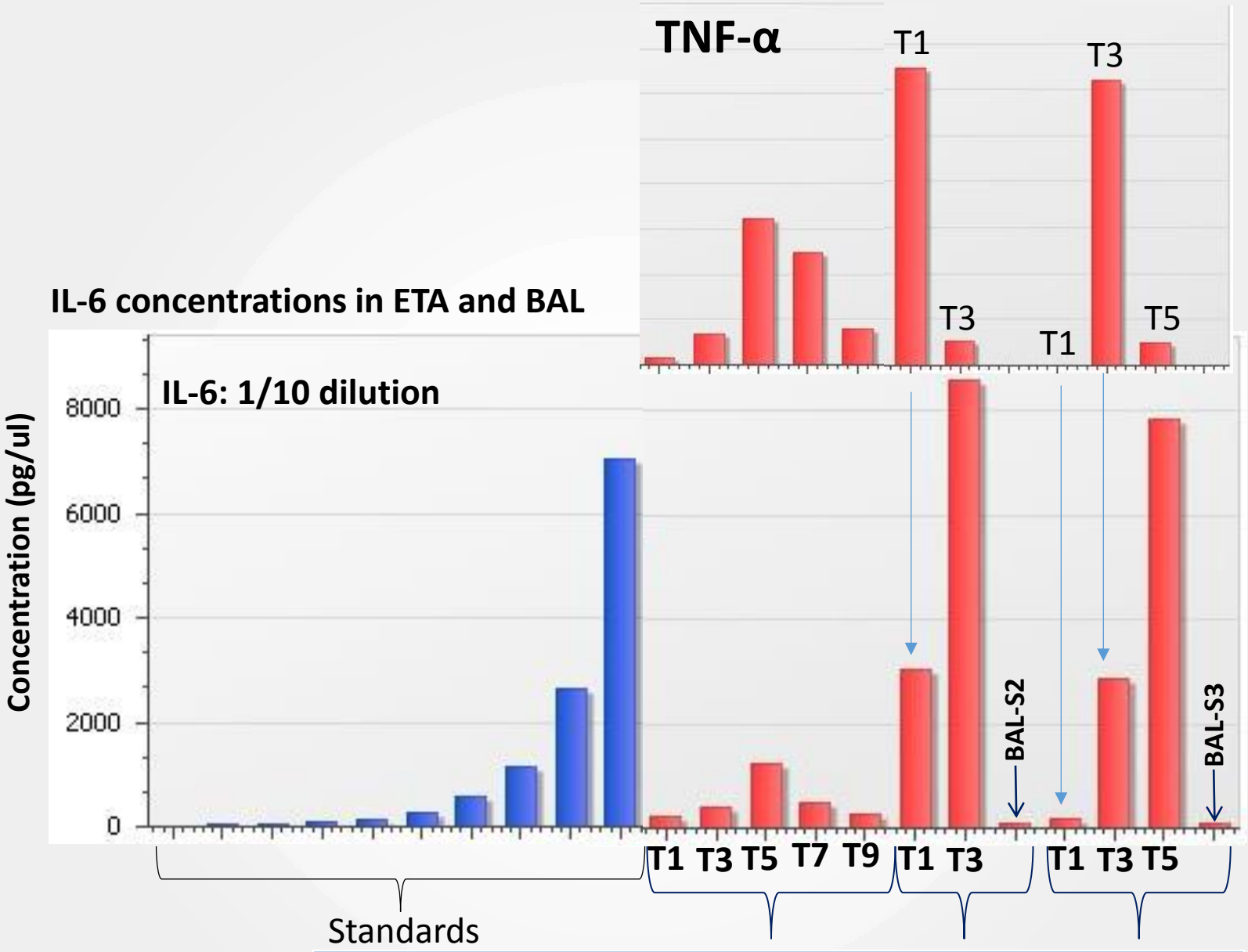
Biospecimens:

- **Endotracheal Aspirate**
- **Bronchoalveolar Lavage (BAL)**

HONORHEALTH

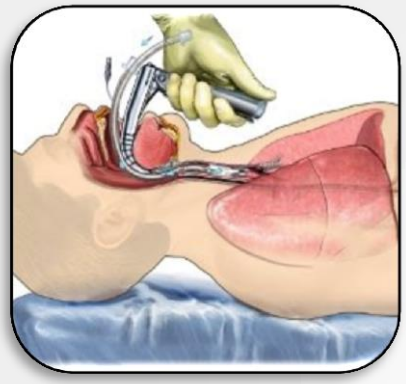
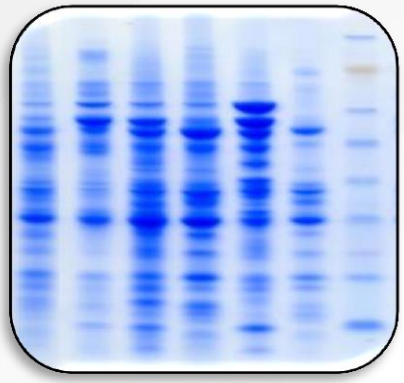
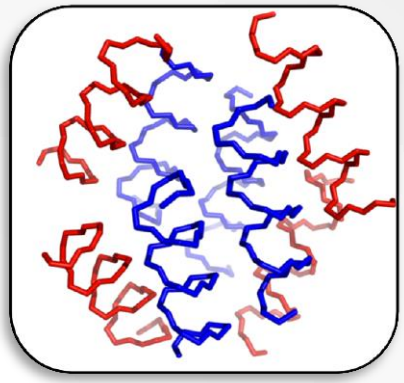
TNF-α concentrations in ETA and BAL biospecimens





Subjects	S1	S2	S3
Dilution factor	1/10	1/10	1/10

Proteomics study: LC-MS/MS

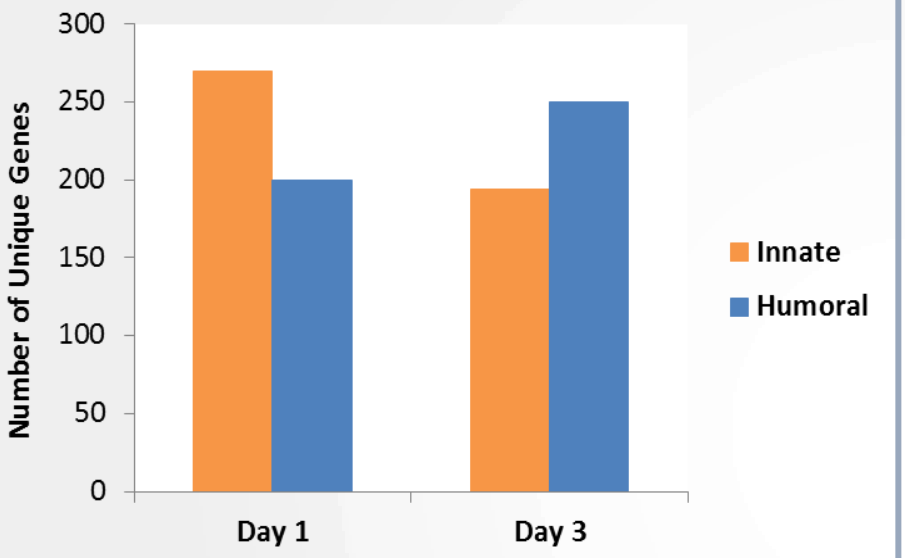





ETA, BAL → Fractionation by SDS-PAGE → Trypsin-Digested Peptides → LC-MS analysis on Thermo LTQ Orbitrap Velos + Waters UPLC

- ETAs of subjects 2 and 3 separated by gel electrophoresis
- Excised gel bands digested using a tryptic in-gel digestion protocol[6]
- Digested peptides analyzed by LC-MS (Waters nanoAcquity UPLC, Thermo LTQ Orbitrap Velos mass spectrometer)
- Database identification using MatrixScience Mascot (Uniprot/Swissprot, 2015)
- Functional gene enrichment analysis (GEA) performed using ToppFun (toppgene.cchmc.org)

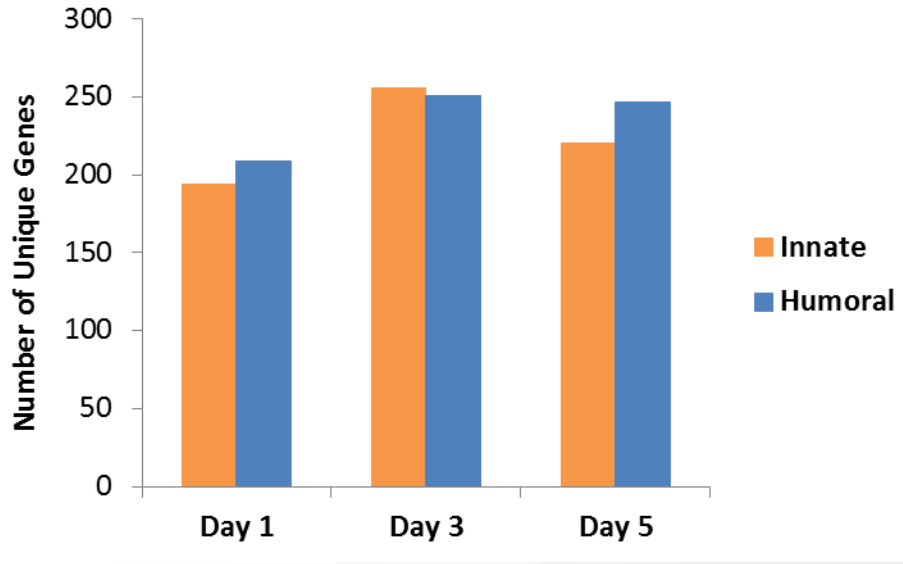
Genes Associated with Immune Responses

Subject 2



- Longitudinal progression from innate immunity to activation of an adaptive immune response.
- Inversed trends suggests shift in homeostatic balance, activation of humoral response, potentially triggered by *Ureaplasma urealyticum* (NGS).

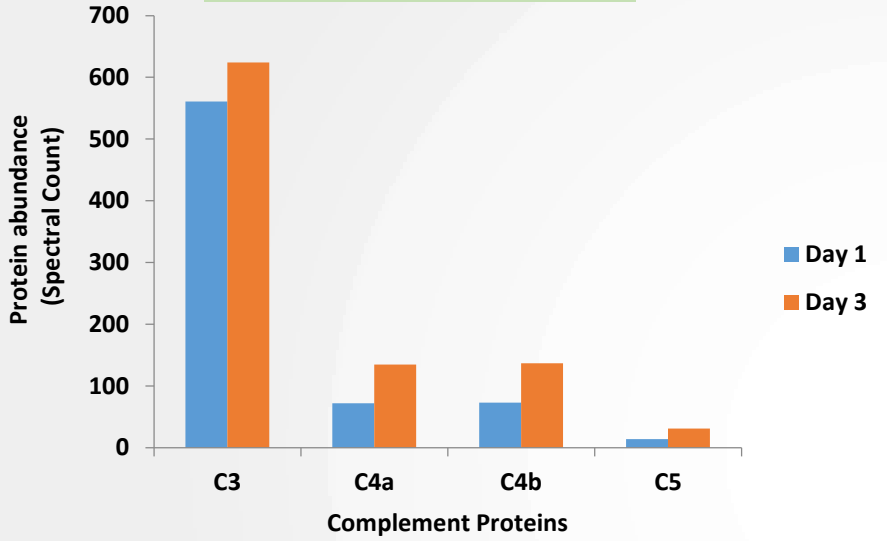
Subject 3



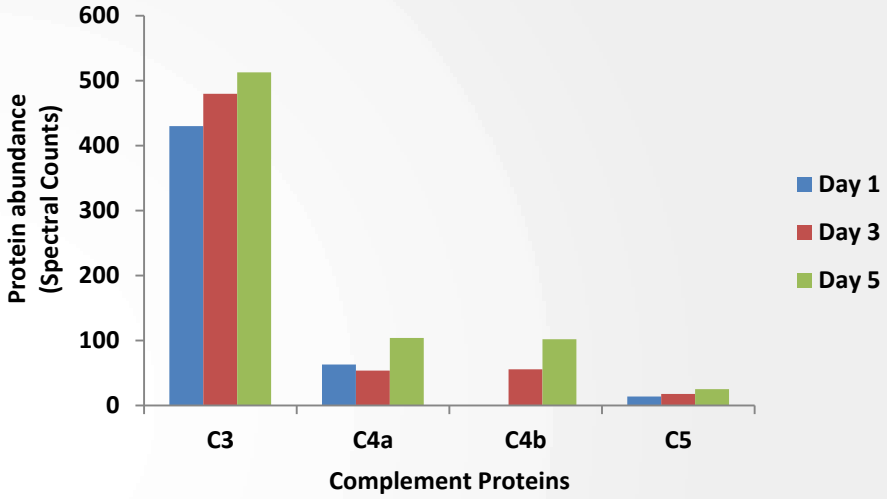
- Gradual increase in humoral response, plateau on day 5
- Innate response increases up to day 3, decrease beyond might be related to antibiotic response.
- Overlapping host responses to infection may be caused by multiple pathogens; *Serratia marcescens* at day 3, and *Peptostreptococcus stomatis* at day 5 (NGS).

Expression of complement proteins

Subject 2



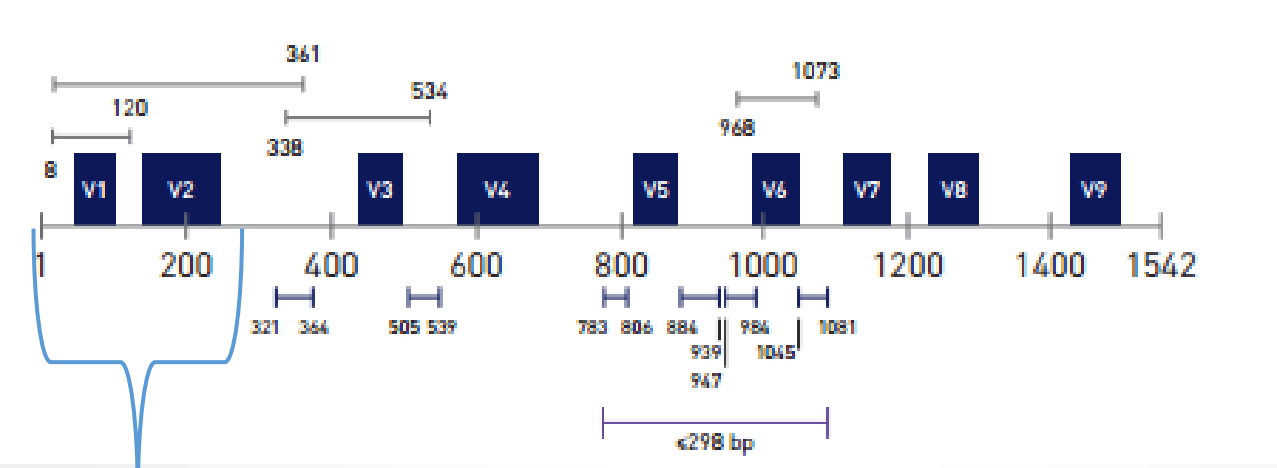
Subject 3



- Increase in expression of complement components C3, C4 and C5 in both subjects highlight complex relationship between innate and humoral immune response
- The complement C4 gene generates to classes of polymorphic protein products (protein cleavage of C4 to C4A (~9kDa) and C4B (~190kDa):
  - C4A high binding affinity to –NH2 groups (peptide antigens) and complement receptor CR1, long half-life, role in immuno-clearance and possibly a link between innate and adaptive responses.
  - C4B faster reaction rate toward carbohydrates and –OH, short half-life and propagates complement activation pathways
  - Absence of C4B during Day 1, but presence of the cleaved C4A may suggest binding to bacterial carbohydrate groups

Diagnostic: Pathogenic Classification

- **16s Ribosomal RNA: Next Generation Sequencing (NGS)**



V1-V2

Subject-1:

T1: Strep spp., Prevotella spp., Neisseria spp., Gemella spp.

T3: Haemophilis spp., Serratia marcescens

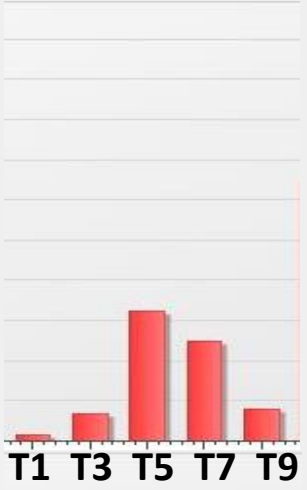
T5: Morococcus cerebrosus, Neisseria lactamica, Fusobacterium nucleatum

T7: Streptococcus anginosus, Prevotella spp., Gemella morbillorum, Fusobacterium necrophorum, Morococcus cerebrosus, *Serratia marcescens (minor)*

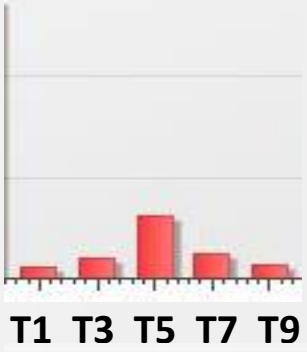
T9: *Serratia marcescens! (nearly all reads are this species)*, Ureaplasma urealyticum

Prediction:
Patient developed Serratia Marcescens pneumonia
Starting on/or around day 11-12,

TNF-α



IL-6
1/10



C5a
1/100

Subject-2:

T1: Prevotella spp., Gemella Haemolysans, Strep spp.

T3: *Ureaplasma urealyticum*! (*nearly all reads are this species*), Mycoplasma hominis

BAL: Neisseria spp., Serratia marcescens, Haemophilus spp., Afipia spp., Bacteroides/Prevotella, Fusobacterium nucleatum, *Ureaplasma urealyticum dominant reads*

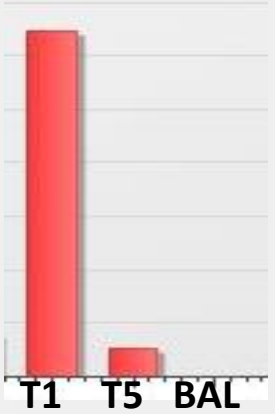
Prediction:

Patient developed Infection/pneumonia from Ureaplasma urealyticum on Day 3,
expected typically in premature newborns.

Clinical Findings: Based on Culture

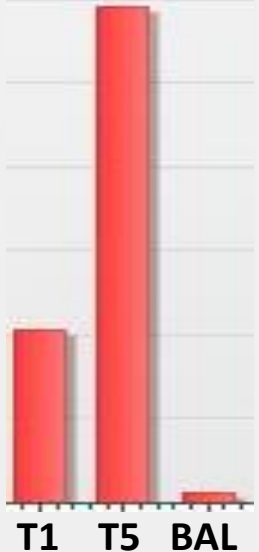
Negative; no organism present

TNF-α



IL-6
1/10

C5a
1/100



Subject-3:

T1: Strep sp.

T3: Prevotella spp., Neisseria spp., Veillonella parvula, Morococcus cerebrosus, Serratia marcescens (minor)

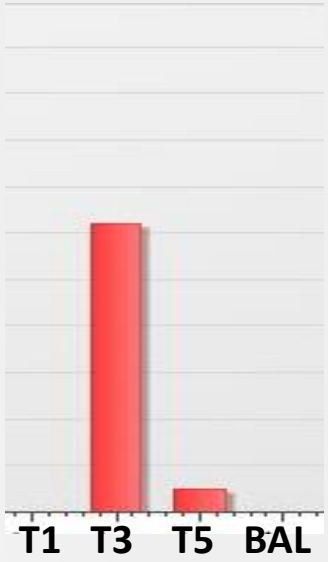
T5: Prevotella spp., Strep spp., Veillonella spp., Haemophilus parainfluenzae,**Peptostreptococcus stomatis** (dominant)

BAL: **Peptostreptococcus stomatis (dominant)**, prevotella spp.

Prediction:
patient developed ***Peptostreptococcus stomatis***
Infection/pneumonia on or about day 5.

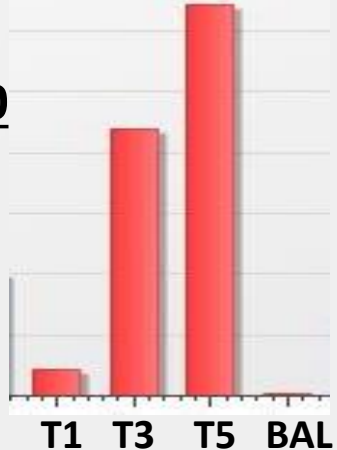
Clinical Findings:
Negative; no organism present

TNF-α



IL-6
1/10

C5a
1/100



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Summary:

- **Defined Immune Mediators, Specific Biological Markers, with Capacity of Early Detection of Infection; 48-72 Hours Prior to Onset of Clinical Symptoms of Pneumonia**

1. **TNF-α secretions Spiked 48-72 hours prior to development of clinical symptoms of pneumonia**
2. **Increased in IL-6 Secretions coincided with the development of clinical symptoms of pneumonia and 48-72 hours post spikes in TNF-α secretion**
3. **The ratio of TNF-α to IL-6 secretion may Provide means for Staging disease progression; early stages of infection to pneumonia development**
4. **Genomic sequencing, 16s rRNA, provided accurate and complete classification of the Invading Pathogen(s)**

- **ETA may Present a non-invasive and easy to access Biospecimen Replacing BAL for the Detection and Diagnosis of Pneumonia infections in Intubated Trauma Patients.**

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