Branched chained amino acids, diabetes and exercise

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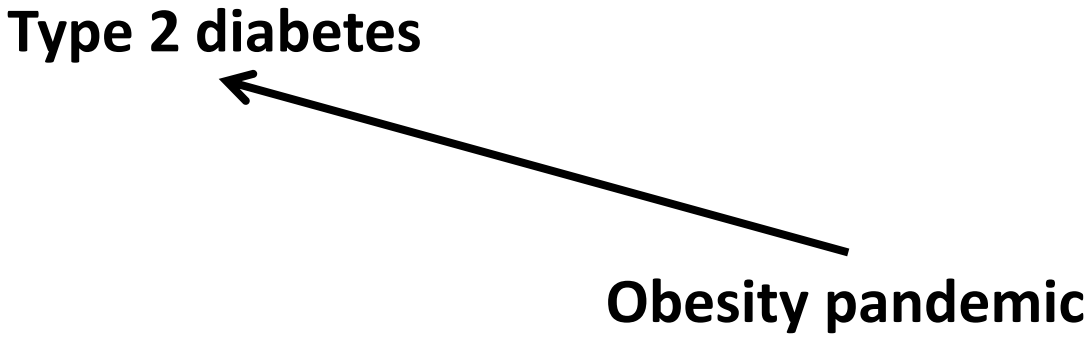
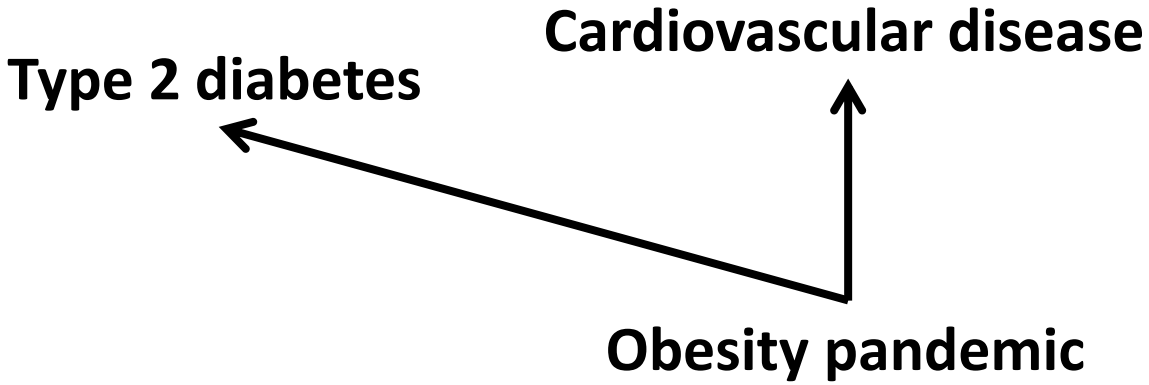
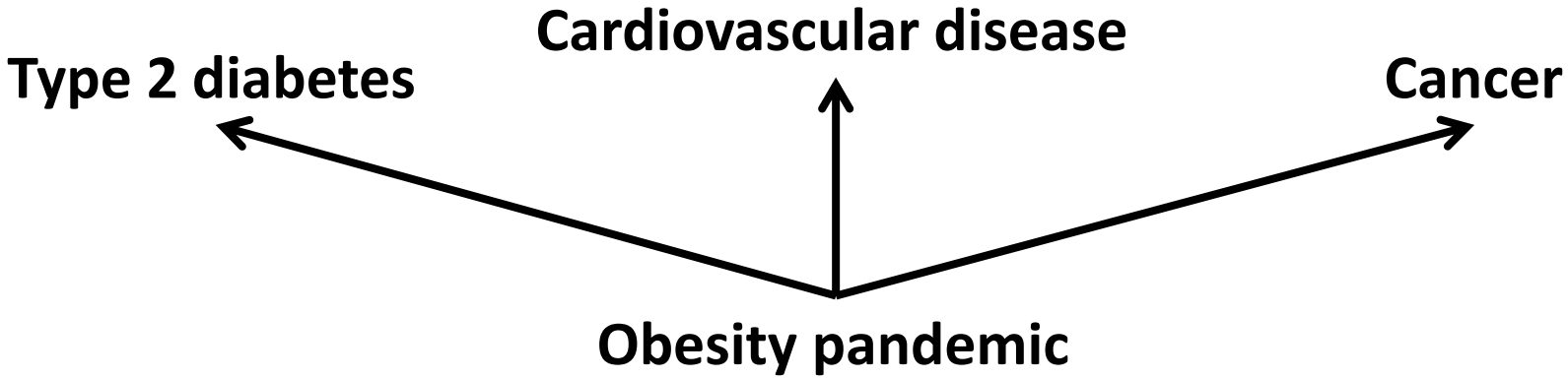
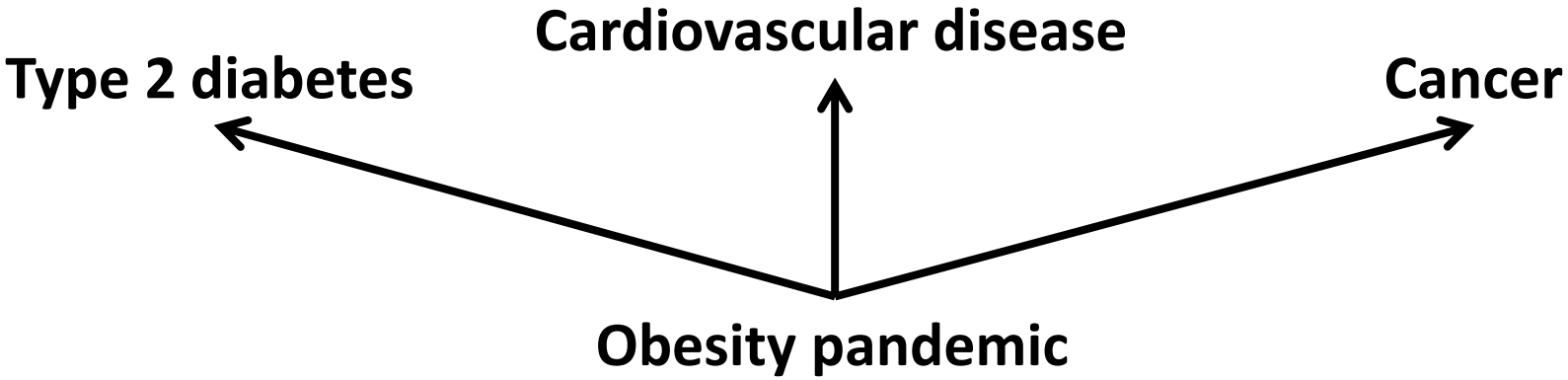
Obesity pandemic


Type 2 diabetes
Obesity pandemic

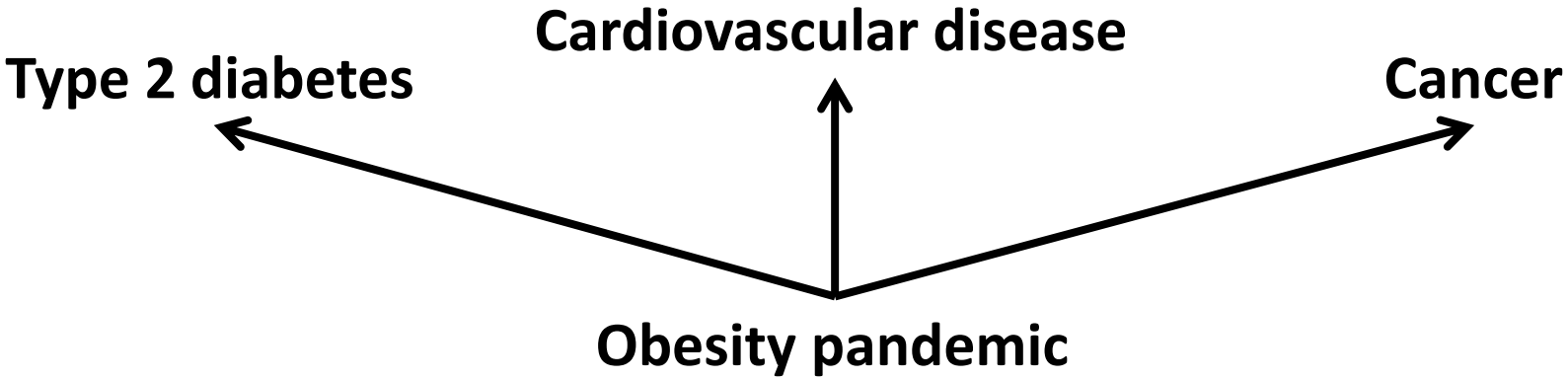

Cardiovascular disease
Type 2 diabetes
Obesity pandemic


Cardiovascular disease
Type 2 diabetes
Cancer
Obesity pandemic



Increased fats in the diet?

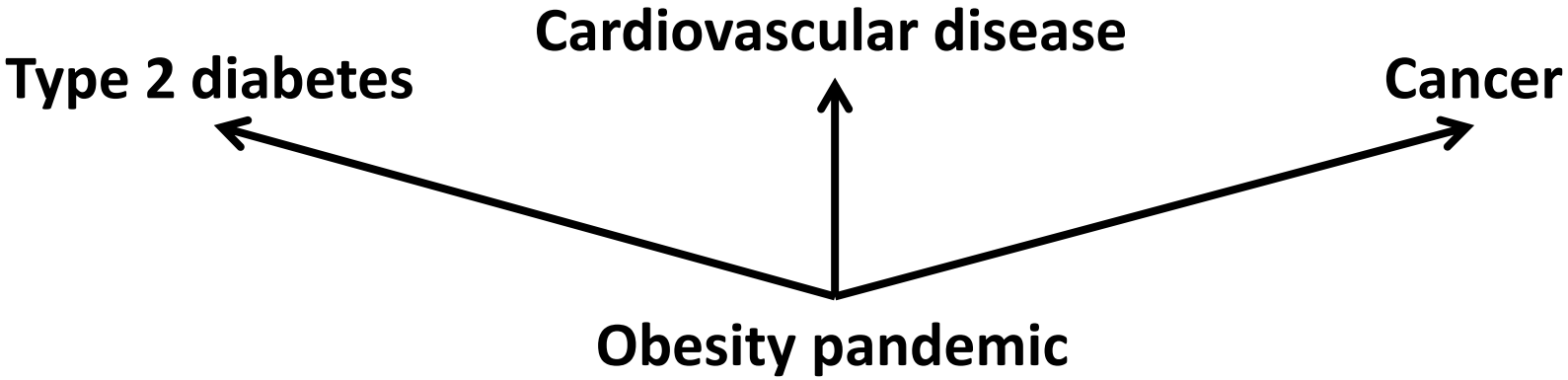
Newgard, 2012

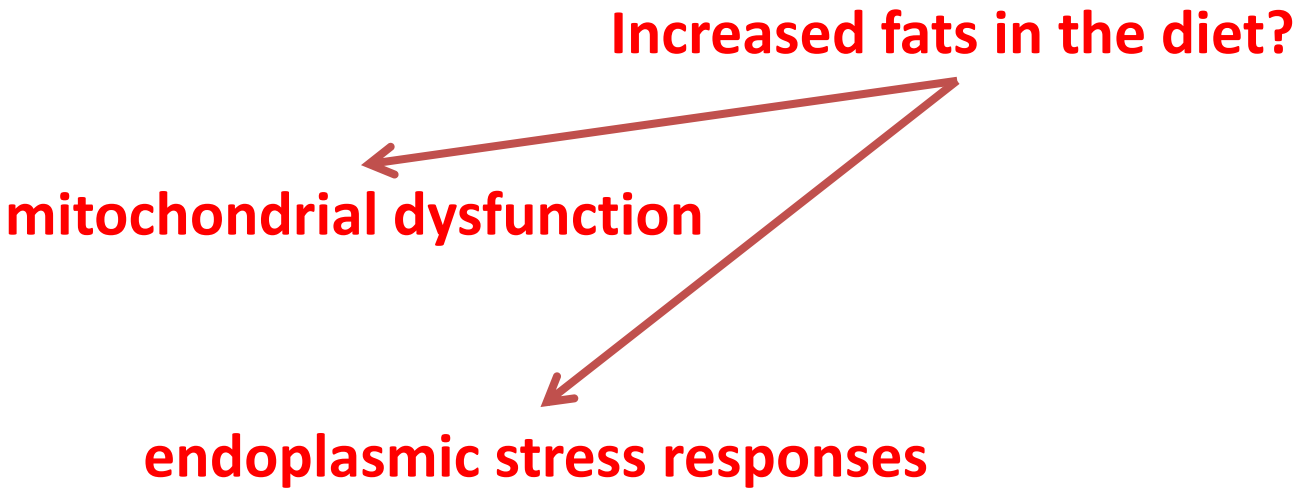


Increased fats in the diet?

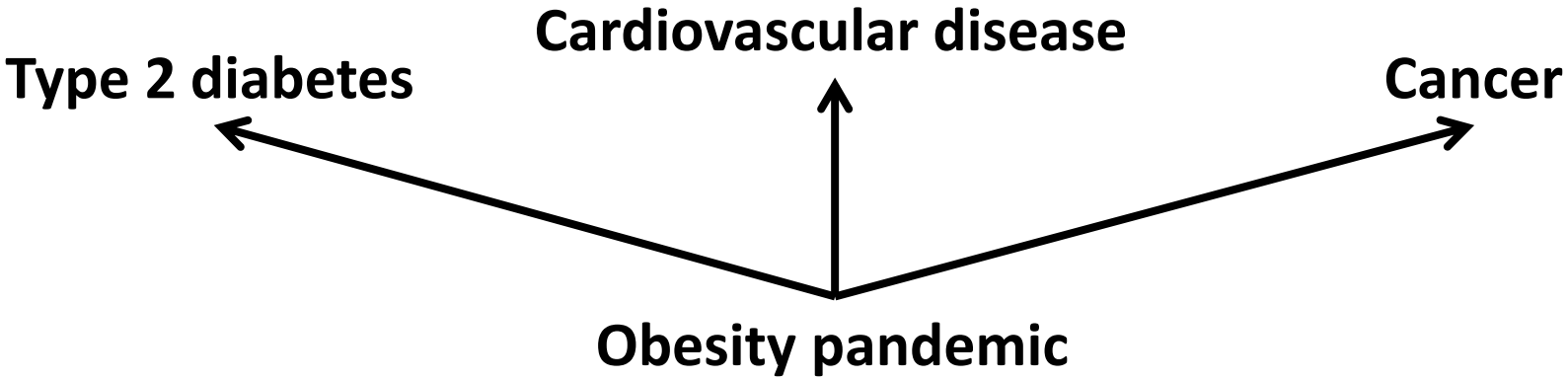
mitochondrial dysfunction

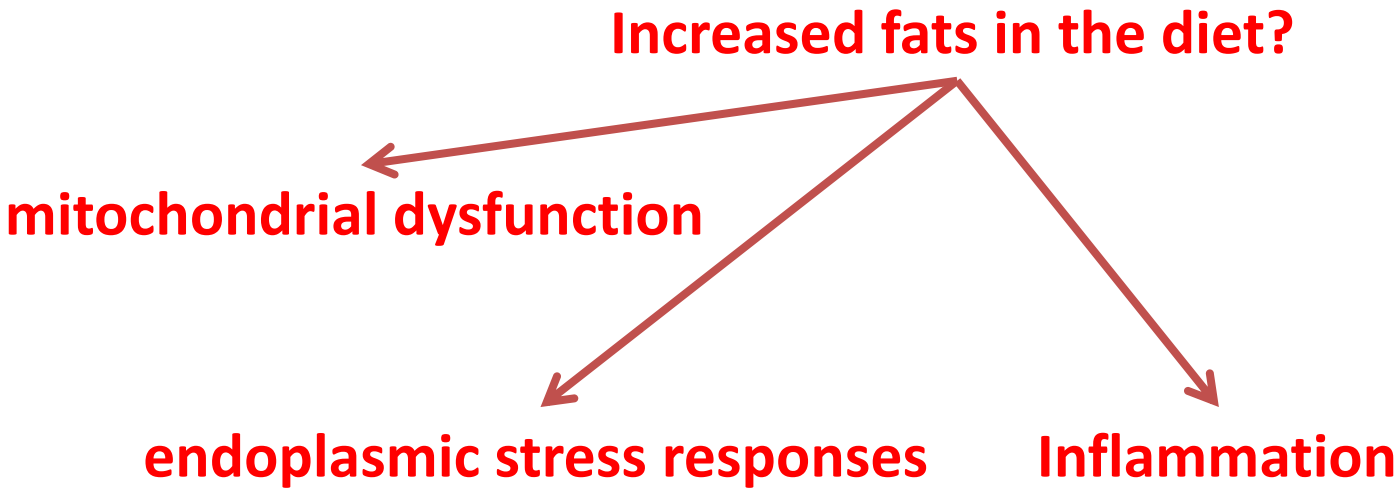
Newgard, 2012



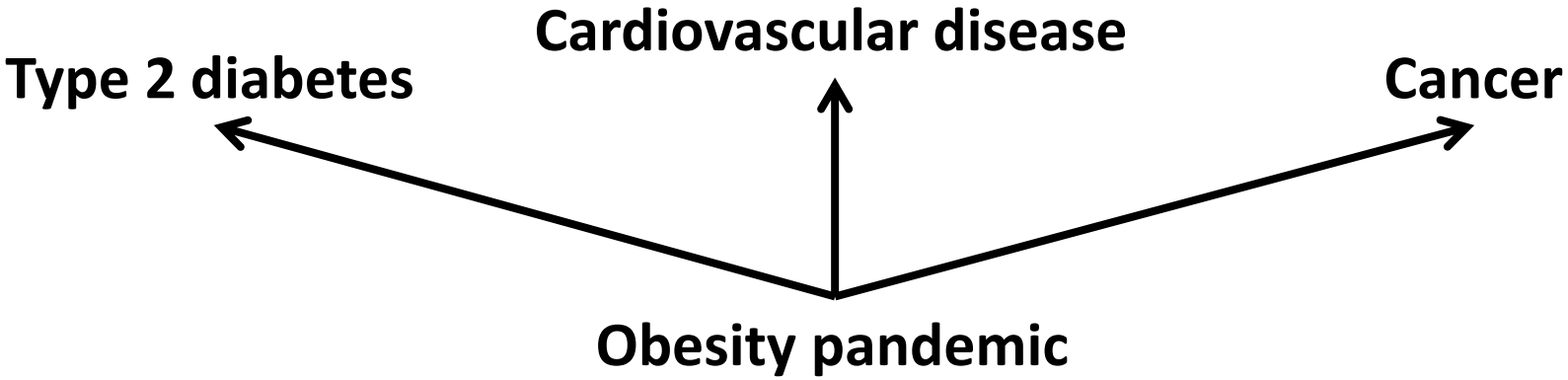


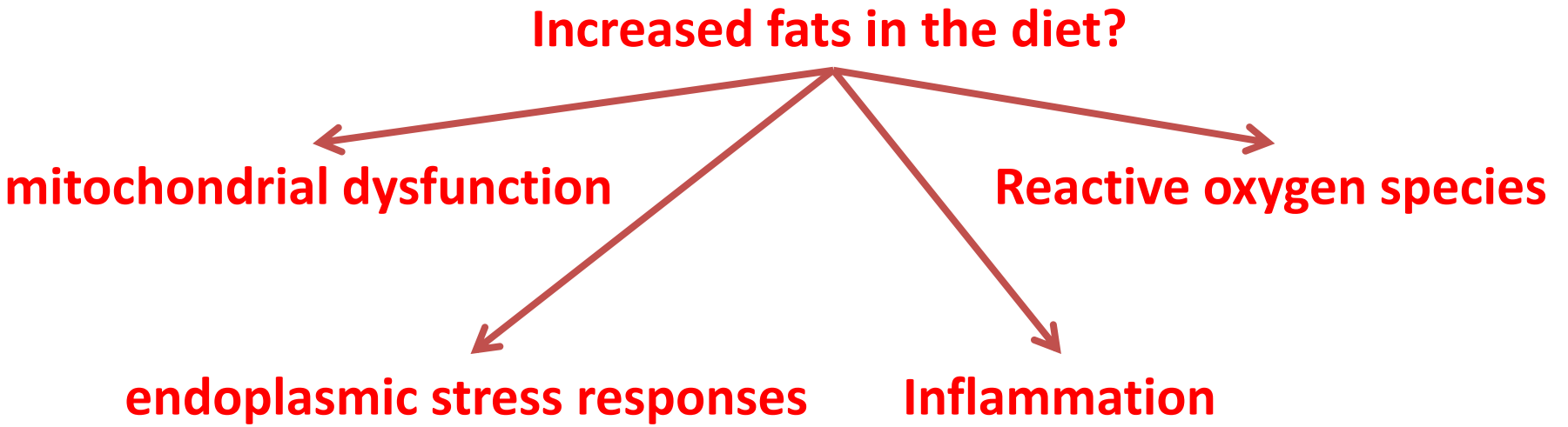
Newgard, 2012





Newgard, 2012

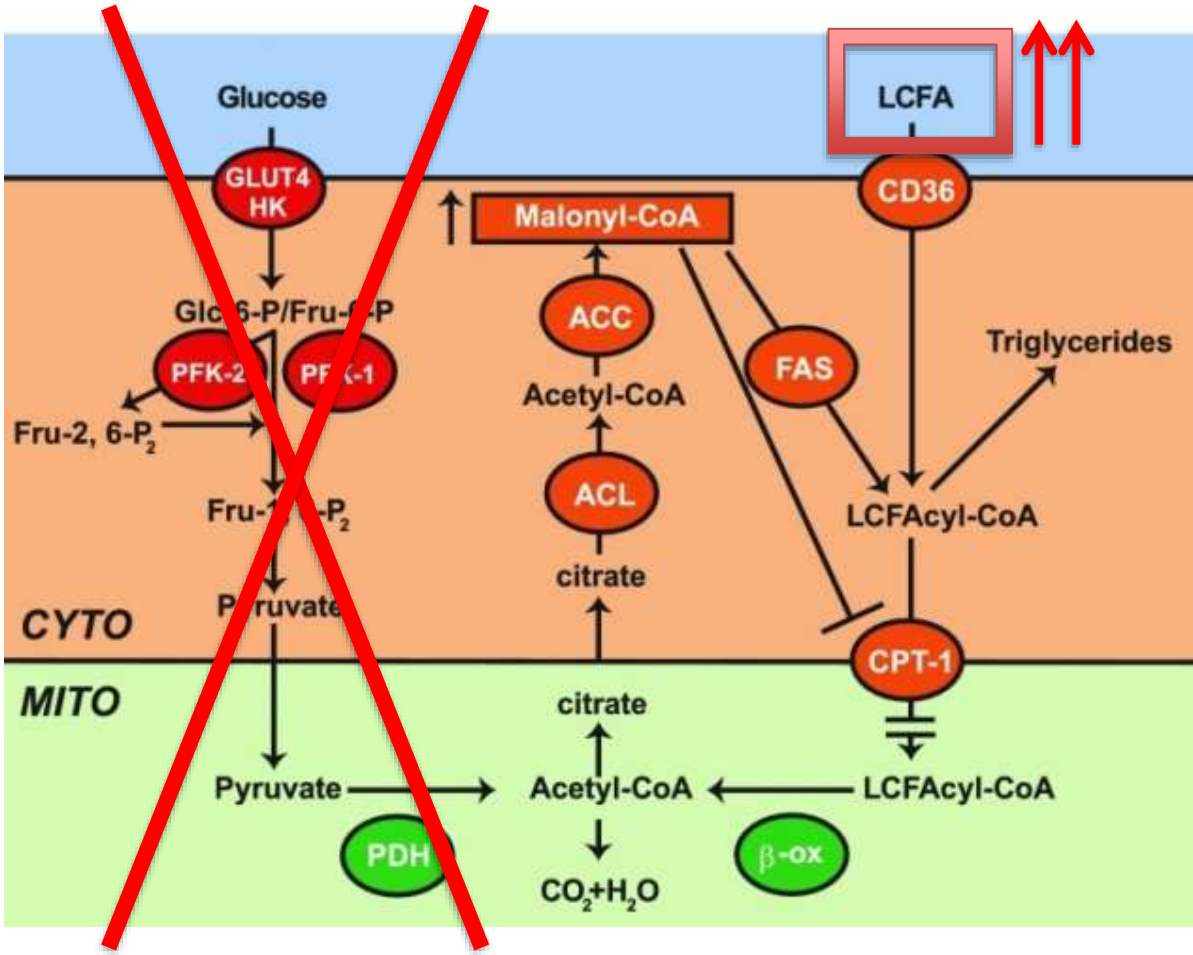




Newgard, 2012

Fat = insulin resistance?

Substrate overflow hypothesis
(Randle cycle)



Randle, 1998; McGarry, 2002

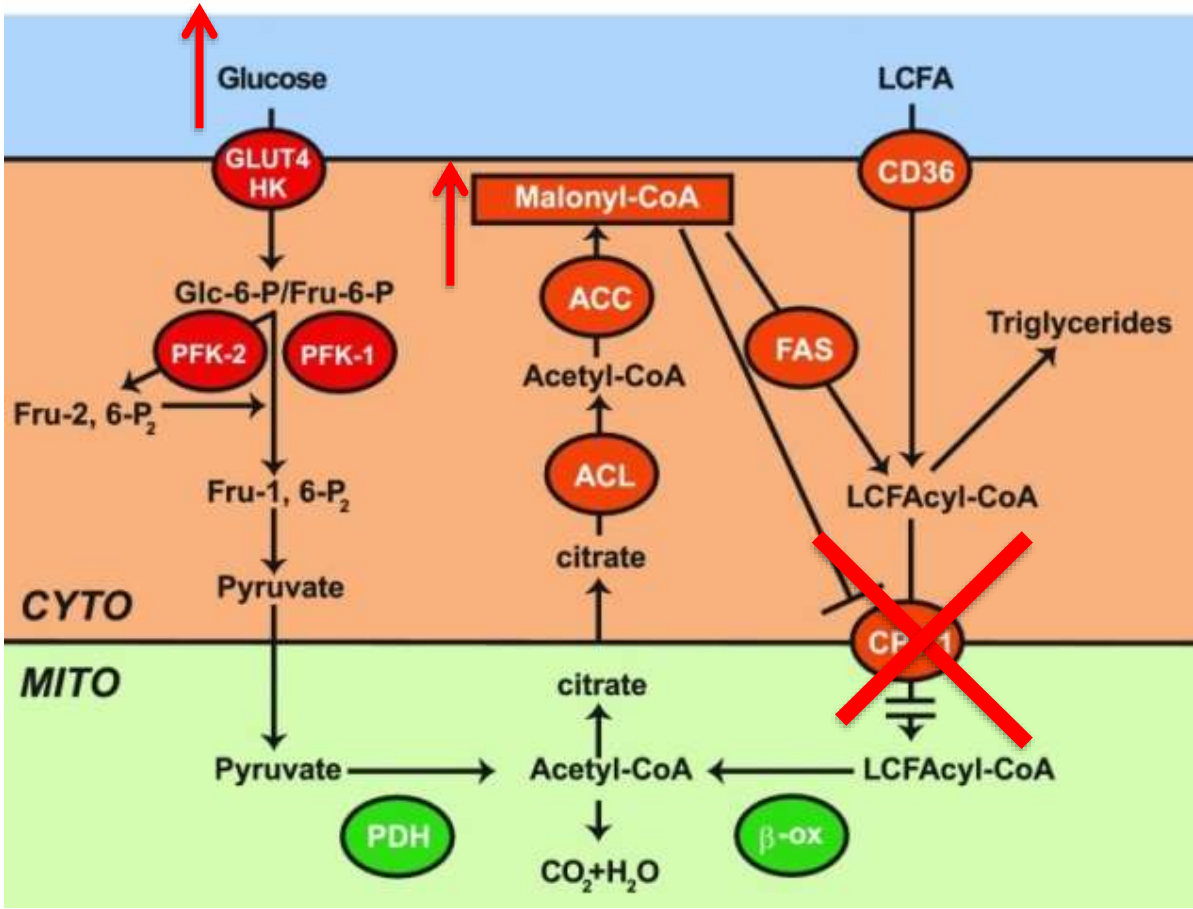
Interaction between glucose and fat metabolism

Fasting:

Glucose < fatty acids

Fed:

Glucose > fatty acids



Randle, 1998; McGarry, 2002

However, this model does not completely explain insulin resistance.

Recent studies suggest role for branched-chain amino acids!

Newgard, 2012
Kelley and Mandarino, 2000

However, this model does not completely explain insulin resistance.

Recent studies suggest role for branched-chain amino acids!

New hypothesize:
BCAA overflow and interference with fat/glucose oxidation

Newgard, 2012
Kelley and Mandarino, 2000

BCAA + insulin resistance

Timeline

1969: Branched chain and aromatic amino acids are elevated in obese humans.

Felig, Marliss, and Cahill, 1969

BCAA + insulin resistance

Timeline

1969: Branched chain and aromatic amino acids are elevated in obese humans.

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.
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(Other focus; muscle growth/maintenance/performance)

Felig, Marliss, and Cahill, 1969

BCAA + insulin resistance

Timeline

1969: Branched chain and aromatic amino acids are elevated in obese humans.

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2005: Animal models

Koves et al., 2005, 2008

BCAA + insulin resistance

Timeline

1969: Branched chain and aromatic amino acids are elevated in obese humans.

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.
.
.
.

2005: Animal models

2009: Advanced metabolomics

Bain et al., 2009

Molecular signatures
Animal models

High-fat

High-fat/BCAA

Standard chow

Koves et al., 2005

Molecular signatures
Animal models

High-fat/BCAA → ***insulin resistant***

Koves et al., 2005

Molecular signatures
Animal models

High-fat/BCAA → ***insulin resistant***

Standard chow + BCAA → normal

Koves et al., 2005

Molecular signatures
Animal models

High-fat/BCAA → ***insulin resistant***

Standard chow + BCAA → normal

High-fat → normal

Koves et al., 2005

Molecular signatures
Animal models

(Similar energy intake, similar weight changes)

Koves et al., 2005

Molecular signatures
Animal models

BCAAs interfere with fat metabolism → linked to insulin resistance
(probably also glucose metabolism..)

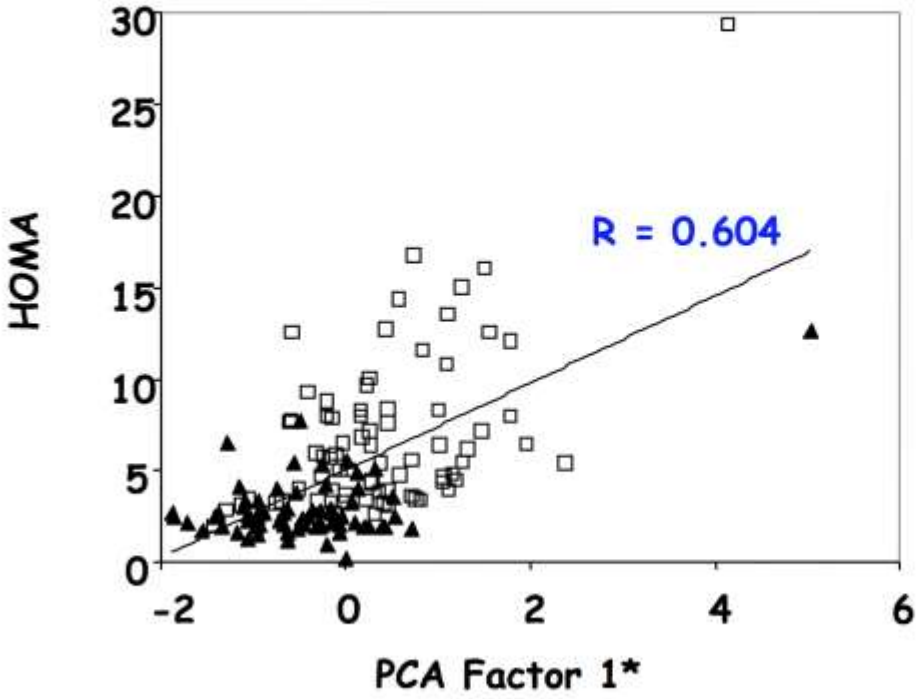
Koves et al., 2005

Molecular signatures
Animal models
Human data

Newgard, 2009

Molecular signatures
Animal models
Human data

Association of a BCAA-Related PCA Factor with Insulin Resistance in Humans



*PCA factor 1 comprised of Val, Leu/Ile, Glx, C3AC, C5AC, Phe, Tyr

Newgard, 2009

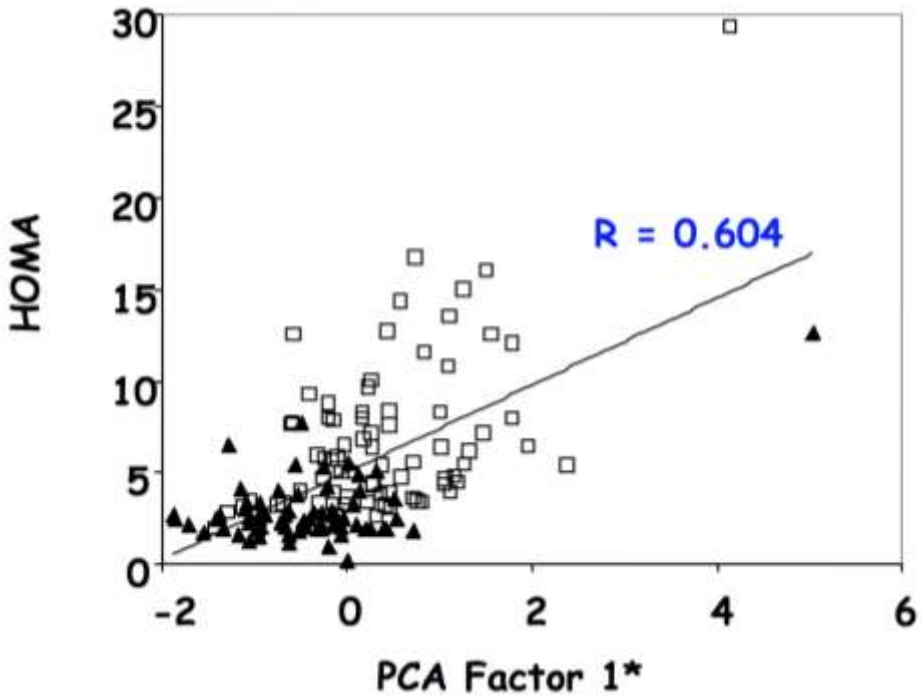
Molecular signatures
Animal models
Human data

Hypothesis

BCAA related to insulin resistance?

Molecular signatures
Animal models
Human data

Hypothesis



Reproduced in 5 studies:

Huffman et al., 2009: Different BMI ranges

Tai et al., 2010: Different ethicities

Shah et al., 2010: Cardiovascular disease

Framingham cohort: Protein intake unimportant

Herman et al 2010: Adipose tissue important

Molecular signatures
Animal models
Human data

Hypothesis

BCAAs and related metabolites

Are strongly associated with insulin resistance and type 2 diabetes (Newgard, et al. *Cell Metabolism* 9: 311, 2009; Huffman, et al. *Diabetes Care* 32: 1678, 2009; Tai, E., et al. *Diabetologia* 53: 757, 2010)

Are prognostic for intervention outcomes (Shah, Svetkey, et al, *Diabetologia* 55: 321, 2011)

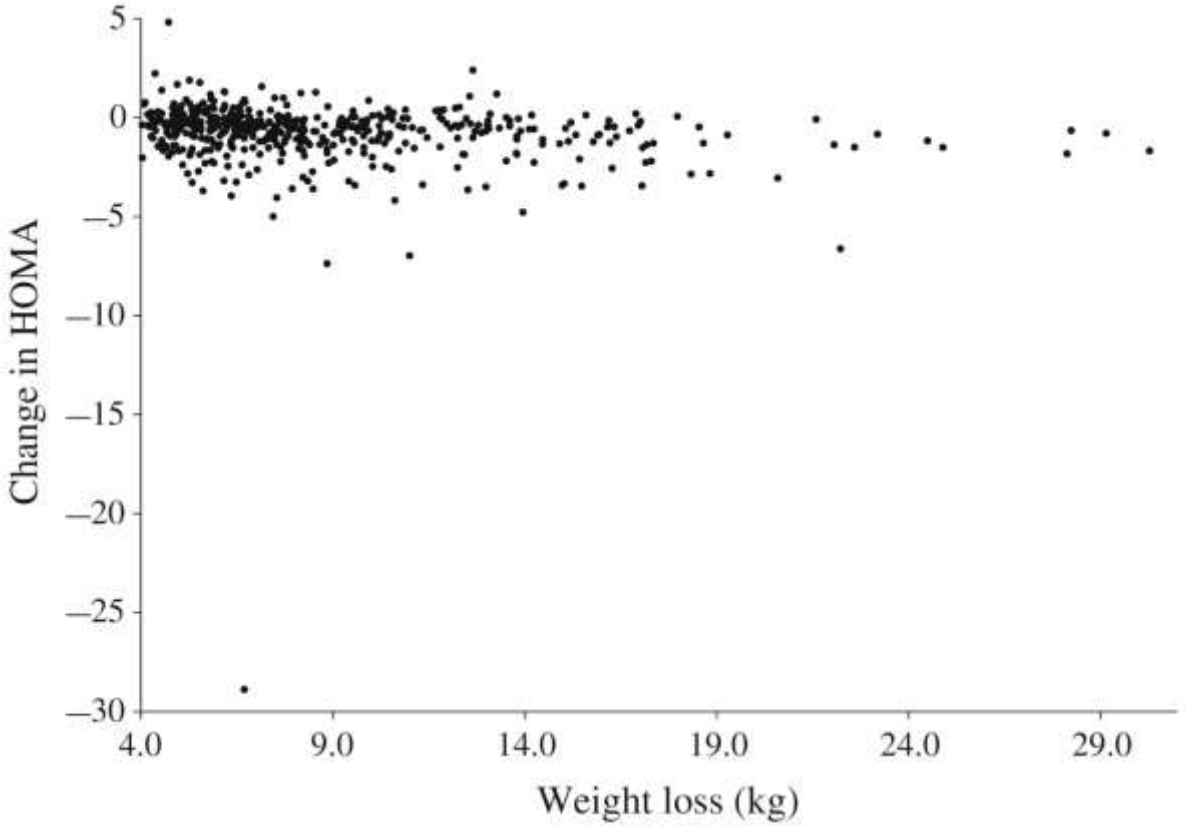
Are prognostic for development of type 2 diabetes (Wang, et al. *Nature Med.* 17: 448, 2011; Palmer, et al. *JCEM* 100: E463, 2015)

Are highly responsive to the most efficacious intervention methods (Laferrere, Svetkey, Newgard, et al. *Science Trans. Med* 3: 80r2e, 2011; Hsiao, et al. *Am. J. Physiol.* 300: E164, 2010; Khoo, et al. *Ann. Surgery* 259: 687, 2014; Glynn, et al. *Diabetologia* 58: 2324, 2015)

Molecular signatures
Animal models
Human data

Hypothesis

6 mo. Weight loss. No association with IR



Shah et al, 2012

Molecular signatures
Animal models
Human data

Hypothesis

Not weight loss, BUT BCAA AND RELATED METABOLITES

Factor	Description	*F* value	Unadjusted *p* value	Adjusted *p* value[a]	Effect size (95% CI)[b]
1	Medium-chain acylcarnitines	0.08	0.78	NS	−0.022 (−0.175, 0.131)
2	Medium-chain dicarboxyl-acylcarnitines	1.96	0.16	NS	−0.109 (−0.262, 0.044)
3	BCAA-related	47.82	<0.0001	<0.0001	−0.513 (−0.659, −0.367)
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	Long-chain dicarboxyl-acylcarnitines	0.32	0.57	NS	−0.044 (−0.197, 0.109)
6	Medium-chain acylcarnitines	3.25	0.07	NS	−0.140 (−0.293, 0.013)
7	Medium-chain acylcarnitines	0.19	0.66	NS	−0.034 (−0.187, 0.119)
8	Short-chain acylcarnitines	3.08	0.08	NS	−0.136 (−0.288, 0.016)
9	Long-chain acylcarnitines	2.06	0.15	NS	0.111 (−0.041, 0.264)
10	Amino acids	4.17	0.04	NS	0.158 (0.006, 0.310)
11	Urea cycle	3.03	0.08	NS	0.135 (−0.018, 0.288)
12	Miscellaneous	0.82	0.37	NS	0.070 (−0.082, 0.223)
13	Miscellaneous	0.11	0.74	NS	0.027 (−0.129, 0.182)

Shah et al, 2012

Molecular signatures
Animal models
Human data

Hypothesis

Large body of indications of a role for BCAAs in insulin resistance

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Flipping the coin:

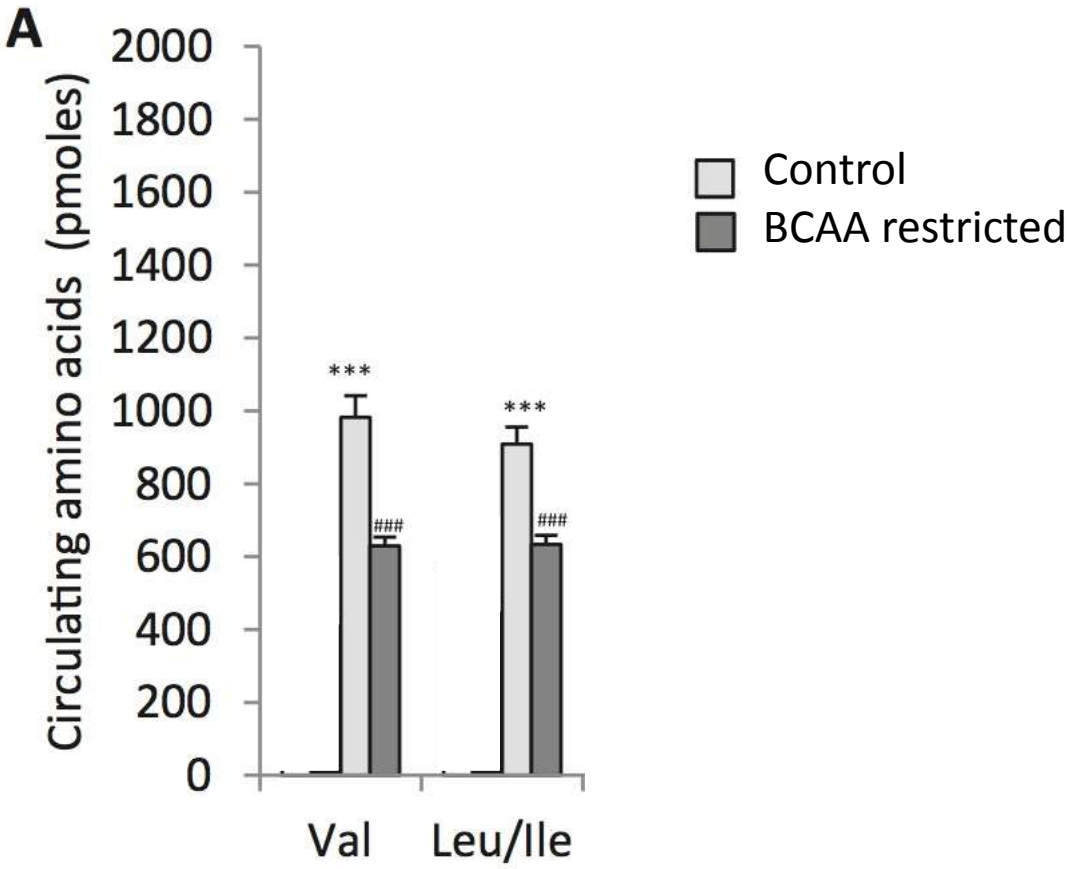
Does BCAA *restriction* improve insulin sensitivity?

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Standard diet vs. BCAA restricted 9 weeks



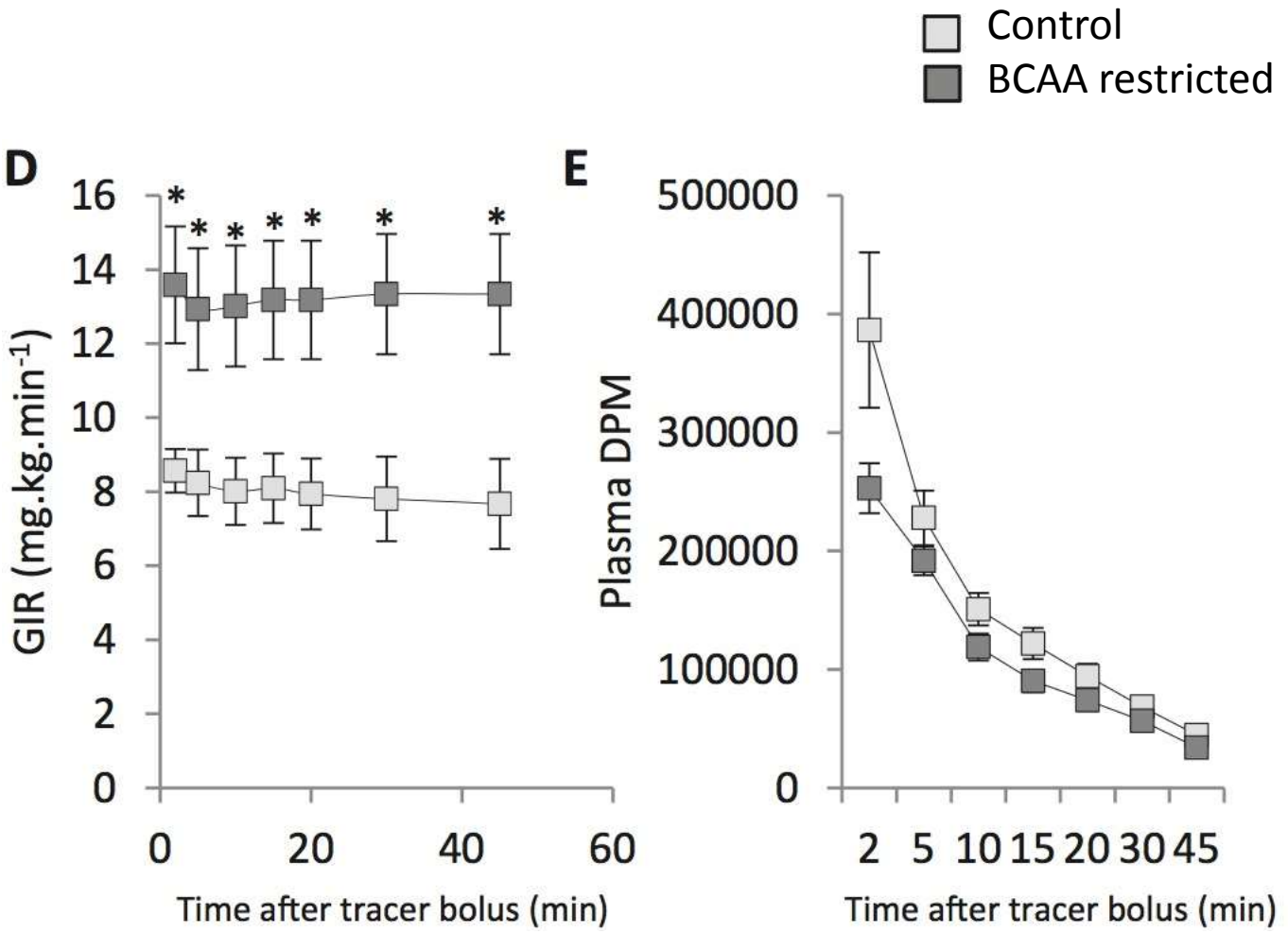
White et al., 2016

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Reduced BCAA increased insulin sensitivity



White et al., 2016

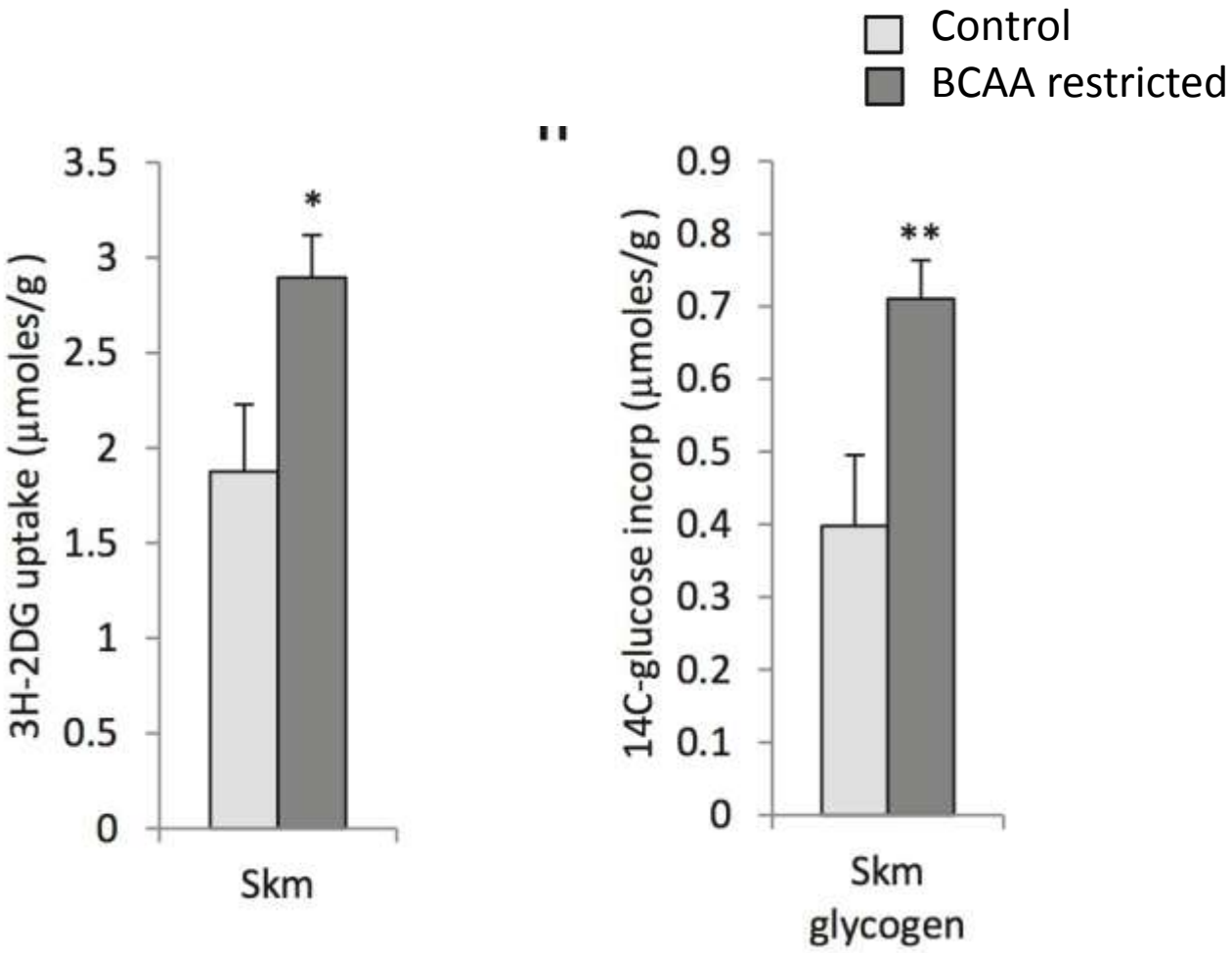
Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Reduced BCAA increased insulin sensitivity

In muscle



White et al., 2016

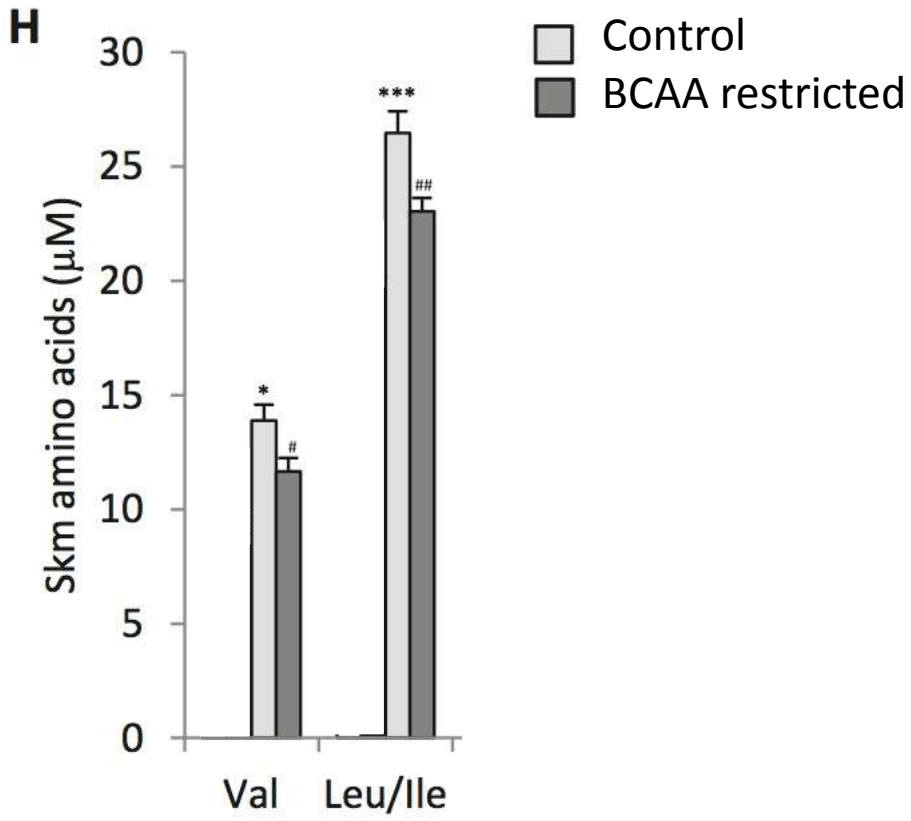
Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Reduced BCAA increased insulin sensitivity

In muscle



White et al., 2016

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Does BCAA restriction improve insulin resistance?

YES!

Less intake
↓
Lower plasma levels
↓
Lower SkM levels
↓
Higher insulin sensitivity

(Possibly by interacting with fat/glucose metabolism, no data..)

White et al., 2016

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Increased levels of BCAA is "bad"
Decreased levels of BCAA is "good"

So, first question:

What causes BCAA to rise in human metabolic diseases?

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Gut microbiota

Essential amino acids

Genetics

Shah et al., 2011

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept


Gut microbiota


Essential amino acids


Genetics

Transplanted feces from obese to lean:
increased BCAA
Reduced insulin sensitivity

Vanessa et al., 2013

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Gut microbiota

Essential amino acids

Genetics

Intake from food

Vanessa et al., 2013

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept


Gut microbiota


Essential amino acids


Genetics

Reduced adipose tissue BCAA catabolism in obesity

Sears et al., 2009, Hsiao et al., 2011

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Gut microbiota

Essential amino acids

Genetics

Increased plasma BCAA levels?

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Gut microbiota

Essential amino acids

Genetics

Increased plasma BCAA levels?

Spill-over to liver and skeletal muscle?

Molecular signatures
Animal models
Human data

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Proof of concept

Gut microbiota

Essential amino acids

Genetics

Increased plasma BCAA levels?

Spill-over to liver and skeletal muscle?

Interference with glucose/fat metabolism

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Gut microbiota

Essential amino acids

Genetics

Increased plasma BCAA levels?

Spill-over to liver and skeletal muscle?

Interference with glucose/fat metabolism

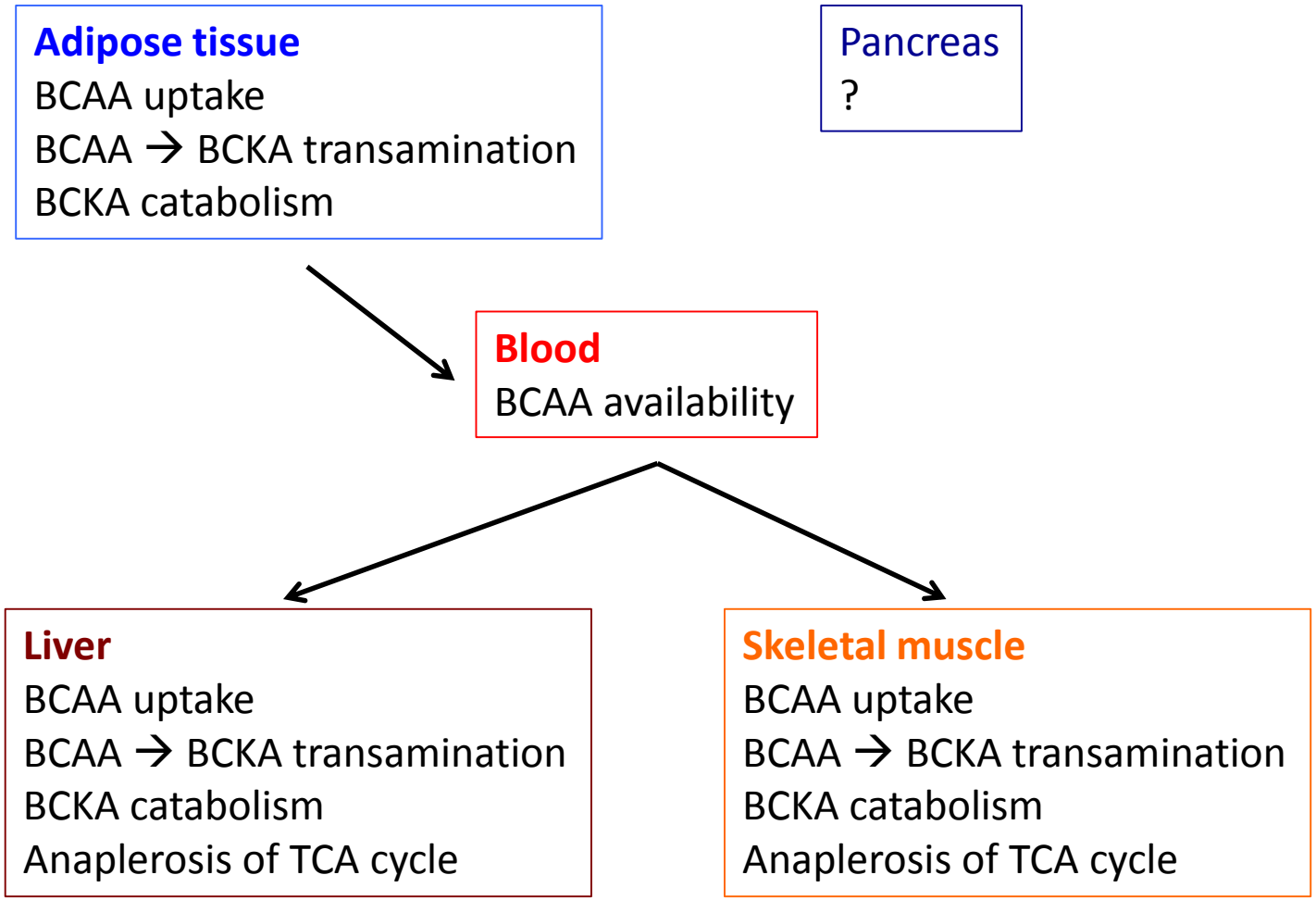
Perturbation of glucose homeostasis

Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Hypothesis of BCAA and insulin resistance: the 4 major tissues in insulin sensitivity



Anaplerosis: BCKAs entering the TCA cycle, competing for the capacity of using Acetyl-CoA from glycolysis.

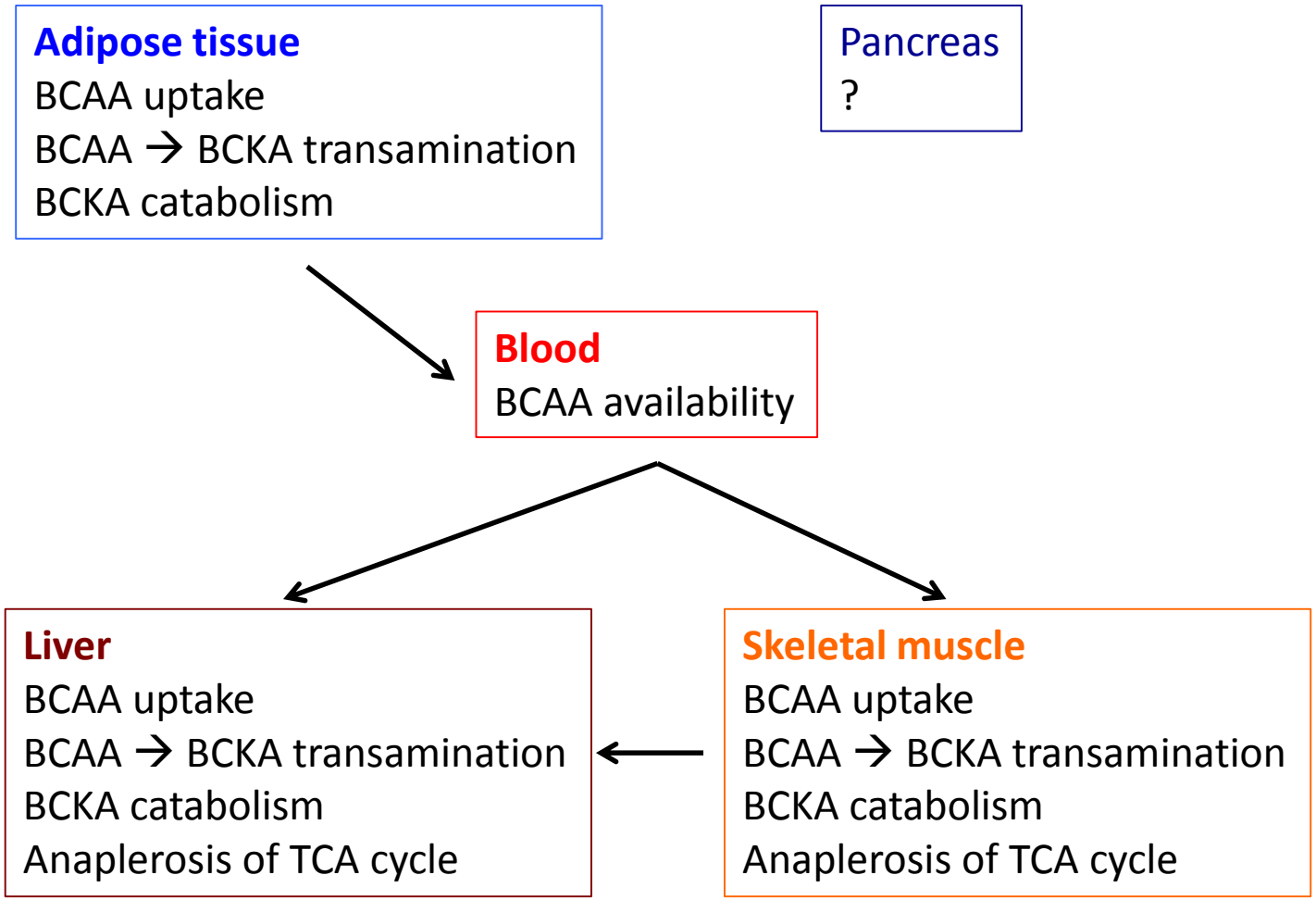
Molecular signatures
Animal models
Human data

Hypothesis

Proof of concept

Test in:
Clinical trails
Cell cultures

Hypothesis of BCAA and insulin resistance: the 3 major tissues



Anaplerosis: BCKAs entering the TCA cycle, competing for the capacity of using Acetyl-CoA from glycolysis.

MyoGlu study: exercise trail and cell cultures

Two groups (13 vs. 13)

Control men:
F-glucose <5.6 mmol/L
and
2 h glucose <7.8 mmol/L
and
BMI 19-25

Pre-diabetic men:
F-glucose ≥5.6 mmol/L
and/or
2 h glucose ≥7.8 mmol/L
and
BMI 27-32

MyoGlu study: exercise trail and cell cultures

Two groups (13 vs. 13)

Control men:
F-glucose <5.6 mmol/L
and
2 h glucose <7.8 mmol/L
and
BMI 19-25

Pre-diabetic men:
F-glucose ≥5.6 mmol/L
and/or
2 h glucose ≥7.8 mmol/L
and
BMI 27-32

Skeletal muscle and adipose tissue biopsies

↓

Global RNA sequencing

↓

Pathway analyses

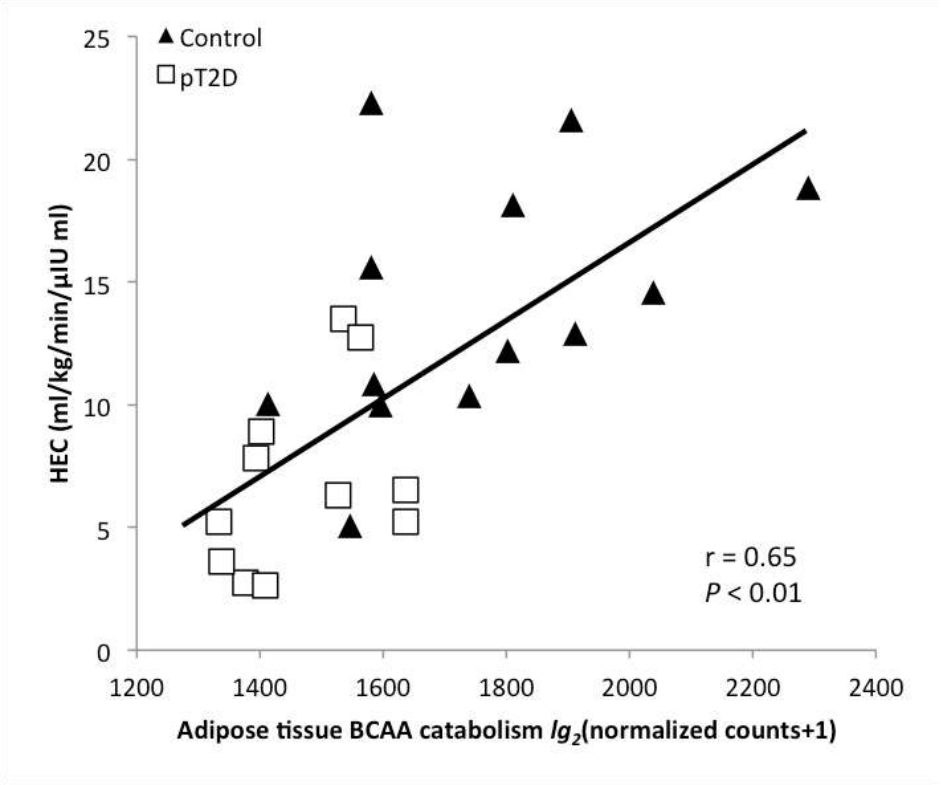
Top 10 down-regulated pathways in pre-diabetes

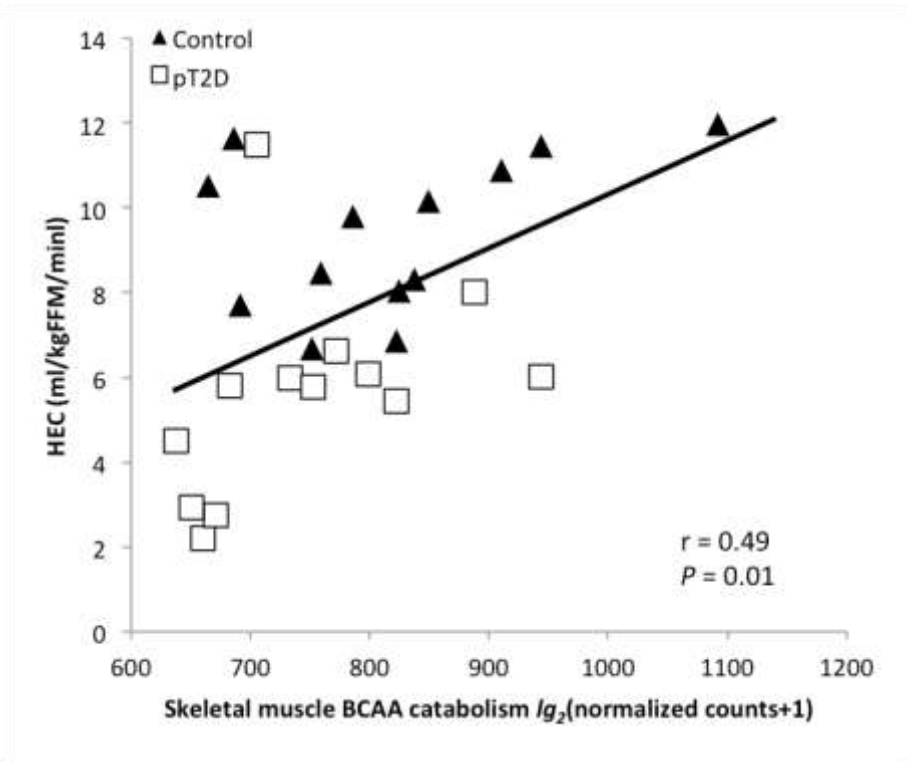
Adipose tissue	Regulation	*P*-values	FDR
hsa00280 Valine, leucine and isoleucine degradation	Down	0,000	0,000
hsa00020 Citrate cycle (TCA cycle)	Down	0,000	0,000
hsa00640 Propanoate metabolism	Down	0,000	0,000
hsa00071 Fatty acid metabolism	Down	0,000	0,008
hsa01040 Biosynthesis of unsaturated fatty acids	Down	0,000	0,016
hsa04740 Olfactory transduction	Down	0,001	0,016
hsa04975 Fat digestion and absorption	Down	0,001	0,019
hsa03013 RNA transport	Down	0,001	0,027
hsa00650 Butanoate metabolism	Down	0,002	0,028
hsa04910 Insulin signaling pathway	Down	0,002	0,041
Skeletal muscle			
hsa03013 RNA transport	Down	0,000	0,003
hsa00280 Valine, leucine and isoleucine degradation	Down	0,000	0,003
hsa00640 Propanoate metabolism	Down	0,000	0,014
hsa00190 Oxidative phosphorylation	Down	0,001	0,027
hsa03010 Ribosome	Down	0,001	0,030
hsa03018 RNA degradation	Down	0,002	0,044
hsa03015 mRNA surveillance pathway	Down	0,002	0,044
hsa03040 Spliceosome	Down	0,002	0,046
hsa03060 Protein export	Down	0,004	0,065
hsa00970 Aminoacyl-tRNA biosynthesis	Down	0,004	0,065

Top 10 down-regulated pathways in pre-diabetes

Adipose tissue	Regulation	*P*-values	FDR
hsa00280 Valine, leucine and isoleucine degradation	Down	0,000	0,000
hsa00020 Citrate cycle (TCA cycle)	Down	0,000	0,000
hsa00640 Propanoate metabolism	Down	0,000	0,000
hsa00071 Fatty acid metabolism	Down	0,000	0,008
hsa01040 Biosynthesis of unsaturated fatty acids	Down	0,000	0,016
hsa04740 Olfactory transduction	Down	0,001	0,016
hsa04975 Fat digestion and absorption	Down	0,001	0,019
hsa03013 RNA transport	Down	0,001	0,027
hsa00650 Butanoate metabolism	Down	0,002	0,028
hsa04910 Insulin signaling pathway	Down	0,002	0,041
Skeletal muscle			
hsa03013 RNA transport	Down	0,000	0,003
hsa00280 Valine, leucine and isoleucine degradation	Down	0,000	0,003
hsa00640 Propanoate metabolism	Down	0,000	0,014
hsa00190 Oxidative phosphorylation	Down	0,001	0,027
hsa03010 Ribosome	Down	0,001	0,030
hsa03018 RNA degradation	Down	0,002	0,044
hsa03015 mRNA surveillance pathway	Down	0,002	0,044
hsa03040 Spliceosome	Down	0,002	0,046
hsa03060 Protein export	Down	0,004	0,065
hsa00970 Aminoacyl-tRNA biosynthesis	Down	0,004	0,065

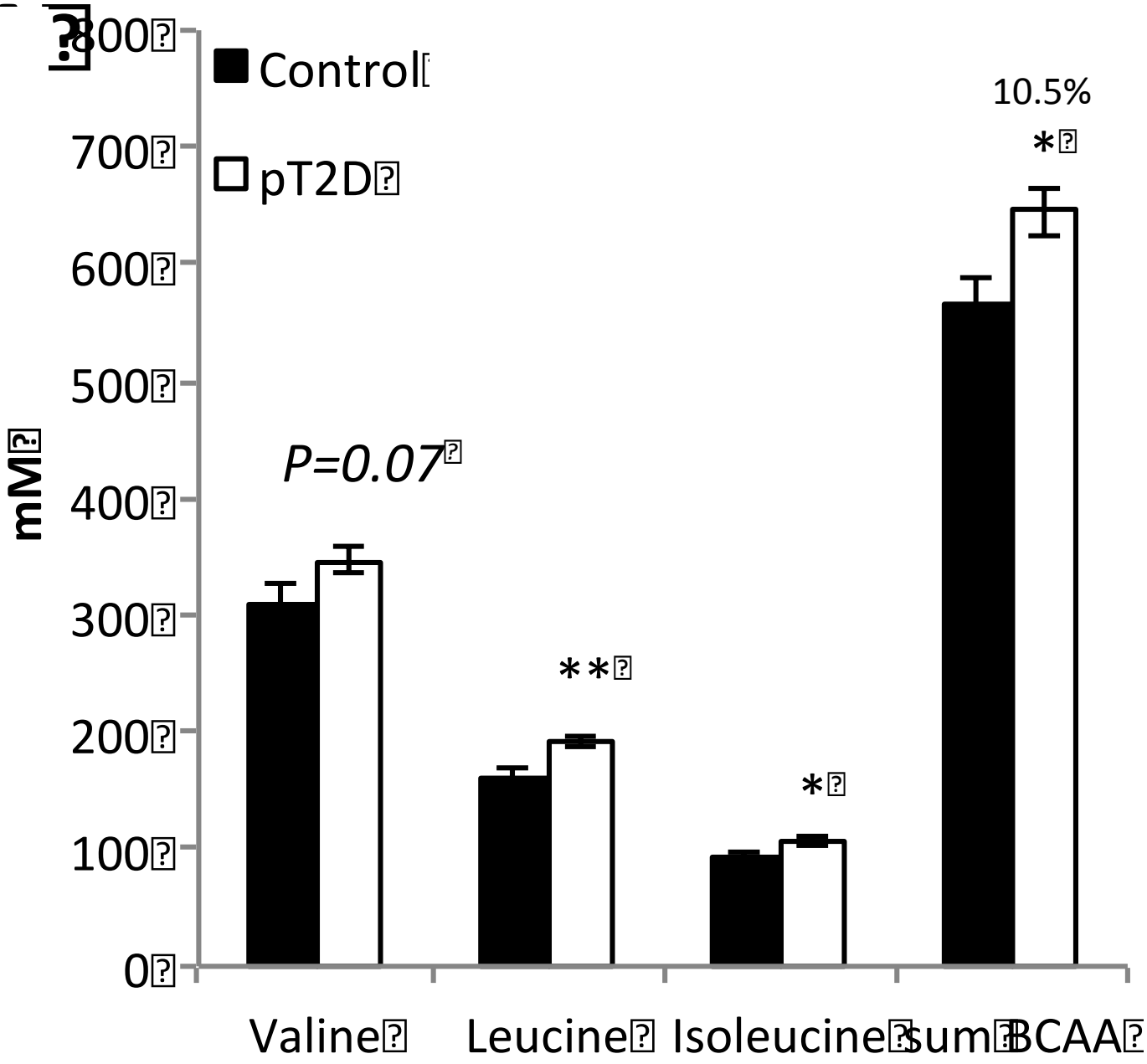
AT & SkM BCAA catabolism correlates with insulin sensitivity





HEC = hyperinsulinemic euglycemic clamp

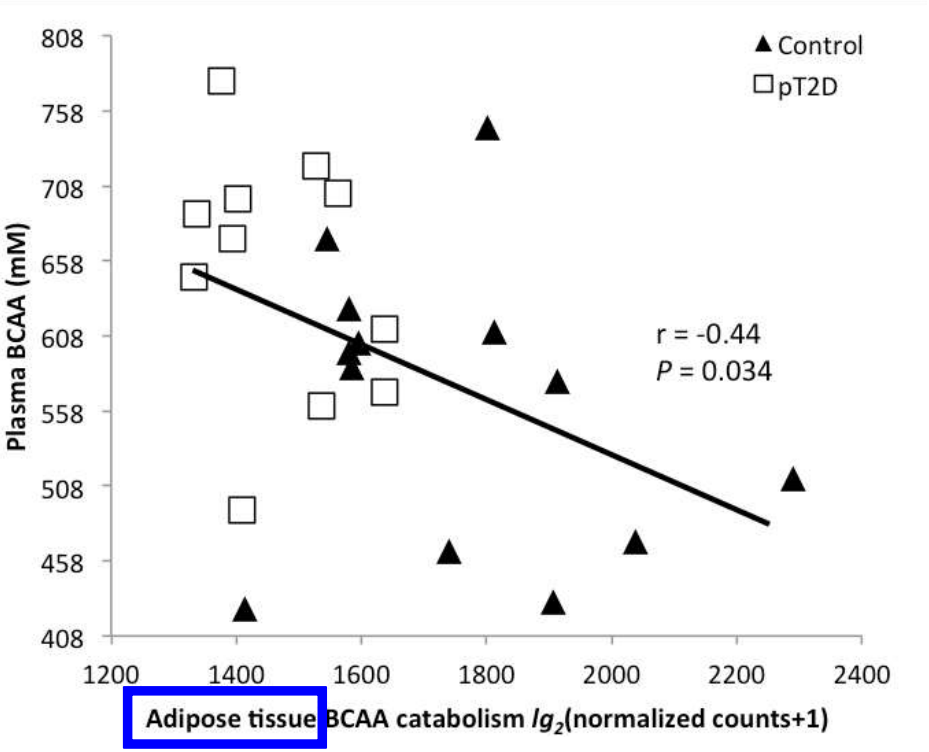
Blood plasma BCAA concentration

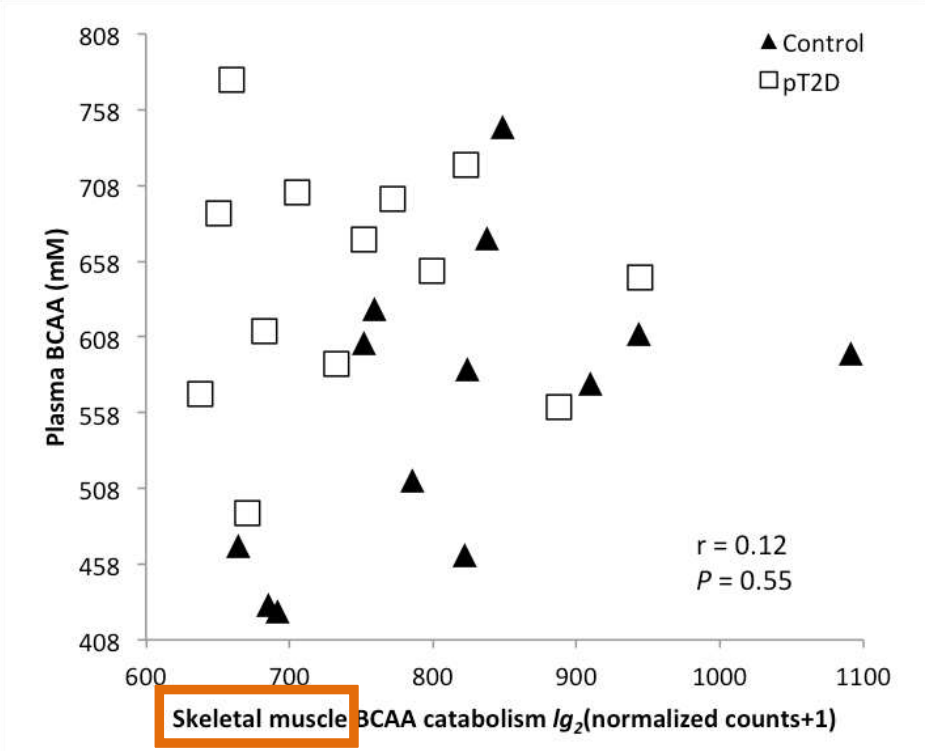


10-20% increase = mitochondrial dysfunction in liver + SkM

Sunny et al 2016

Why elevated plasma BCAA concentrations?

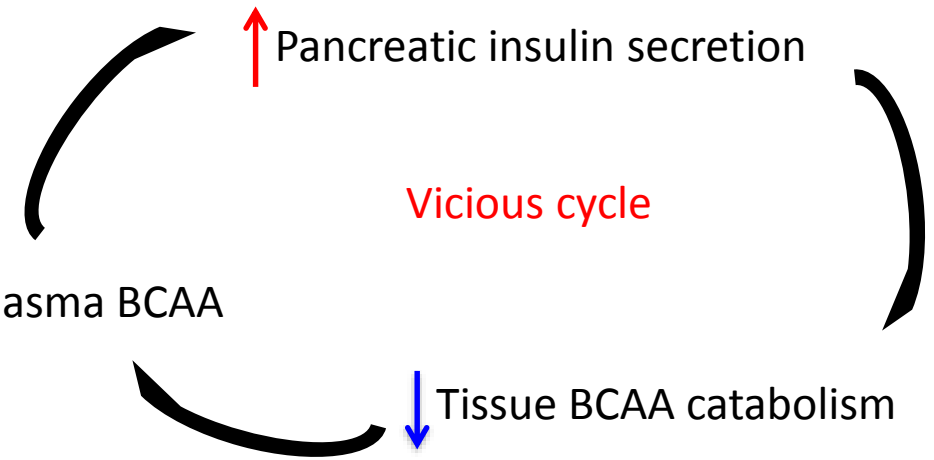


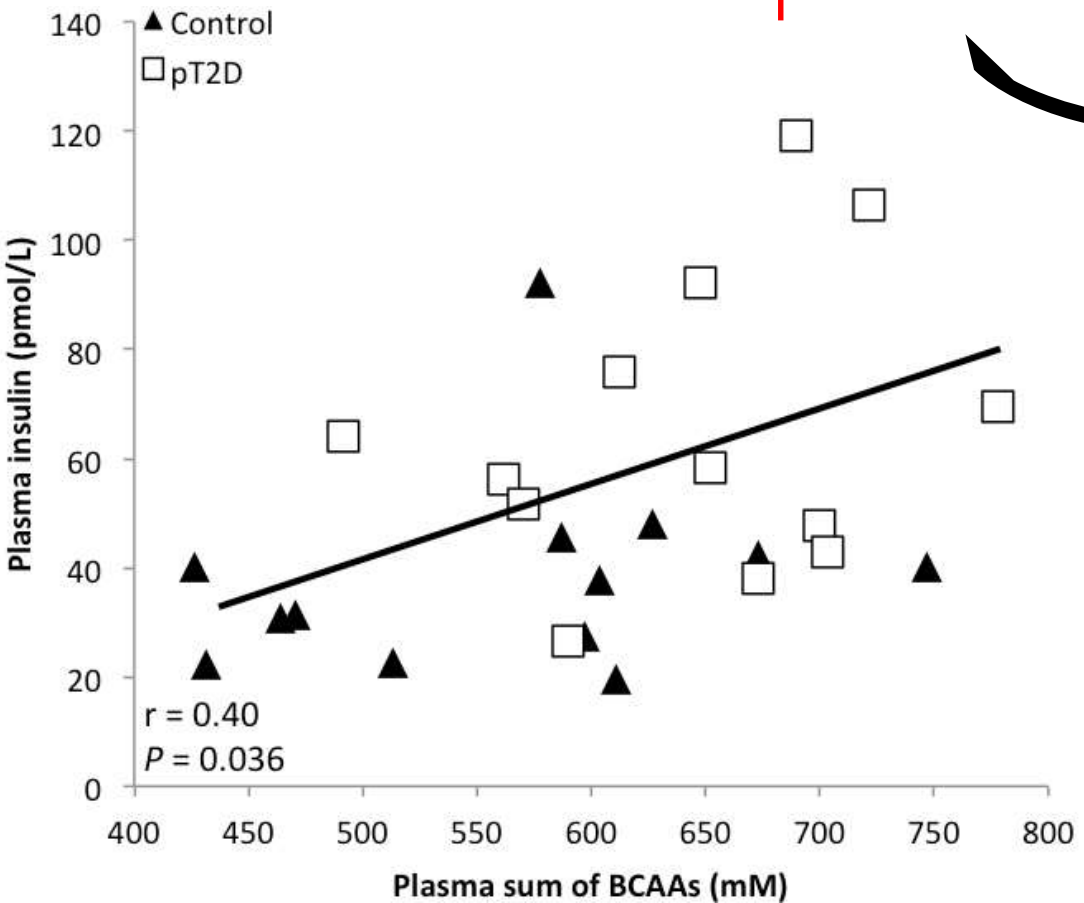


Adipose tissue, not skeletal muscle BCAA catabolism, influences plasma BCAA levels?

Wiklund et al 2016
Guilherme et al 2008
Kahn et al 2006

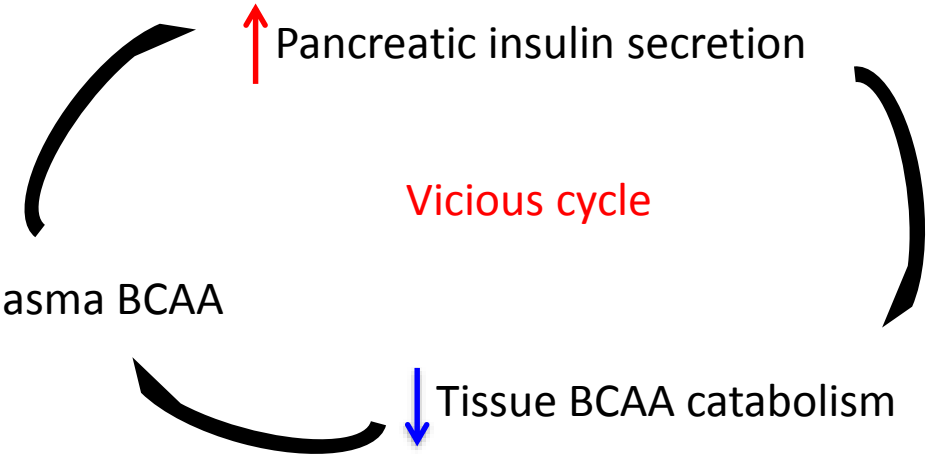
Why elevated plasma BCAA concentrations?

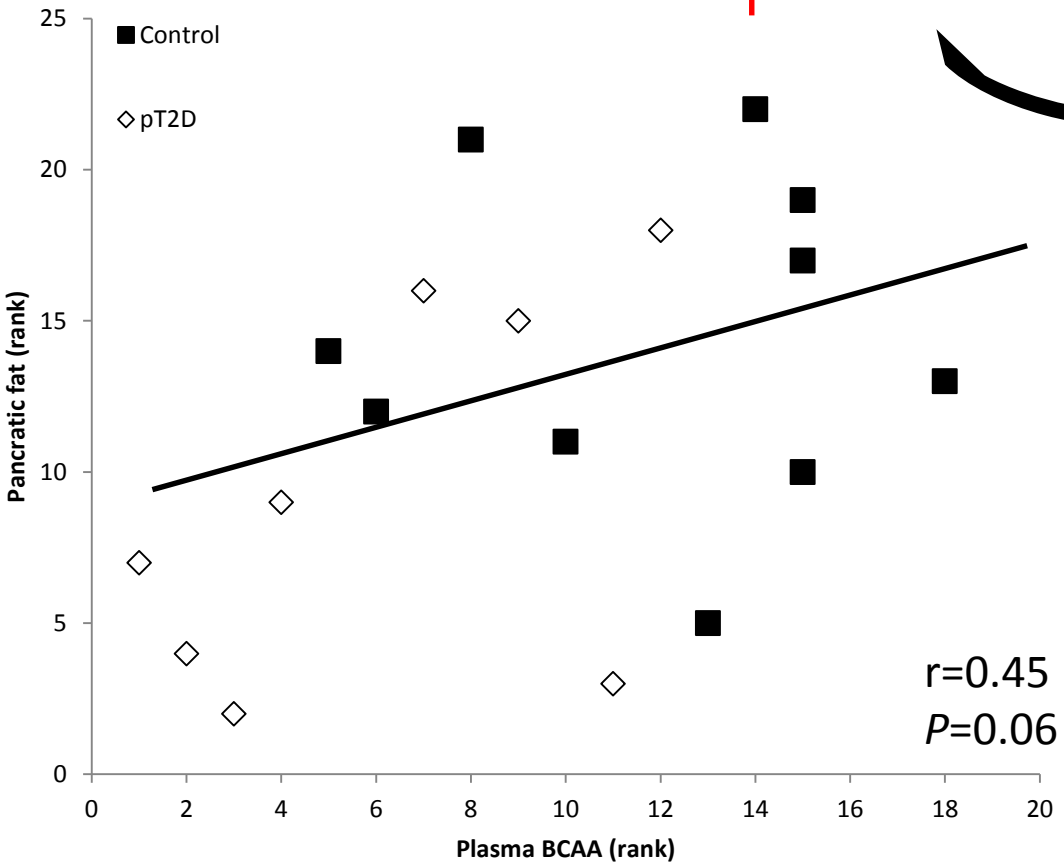




Sunny et al 2016

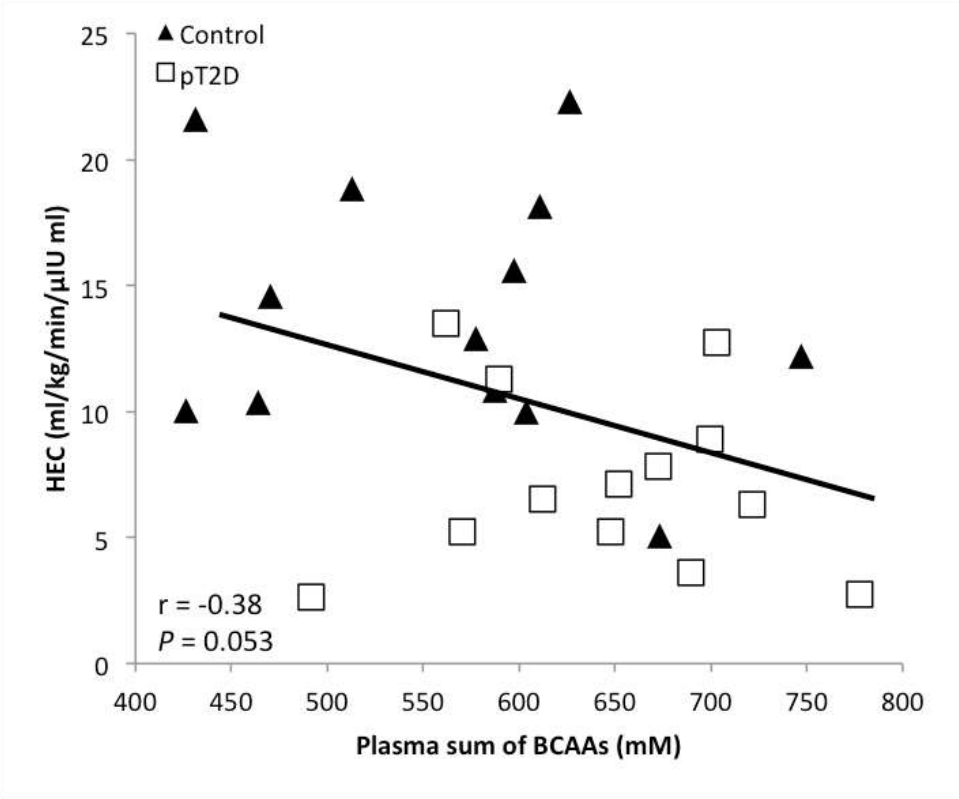
Why elevated plasma BCAA concentrations?

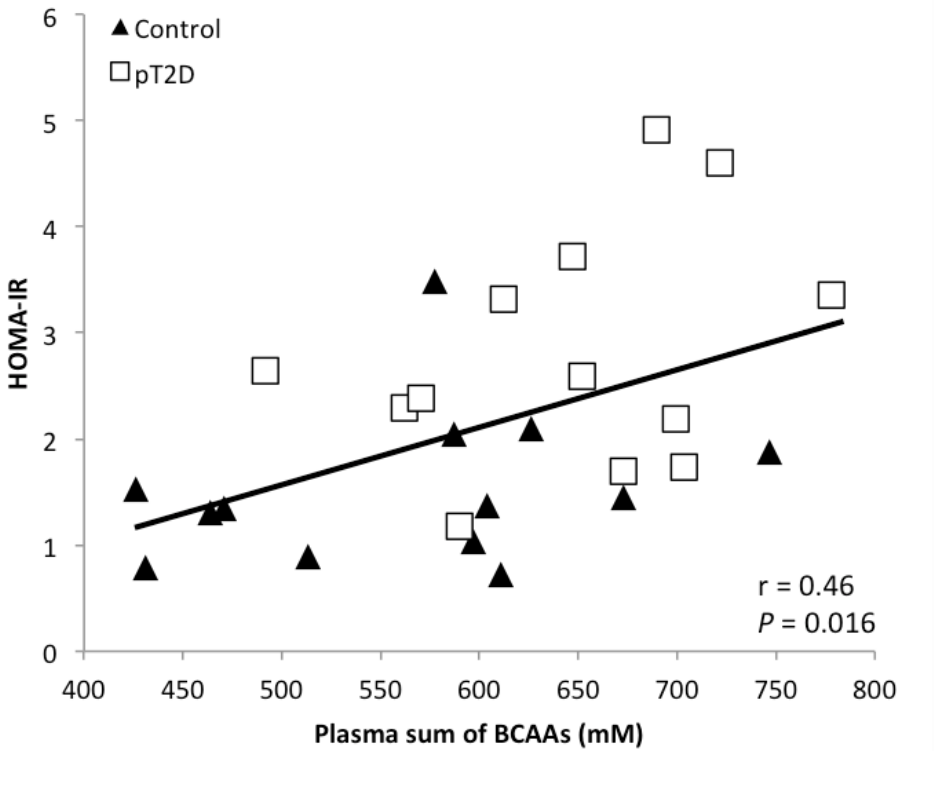




Sunny et al 2016

Consequences of elevated plasma BCAA concentrations

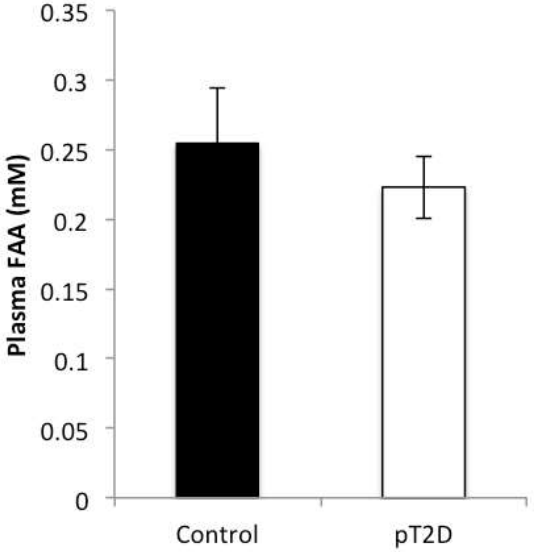


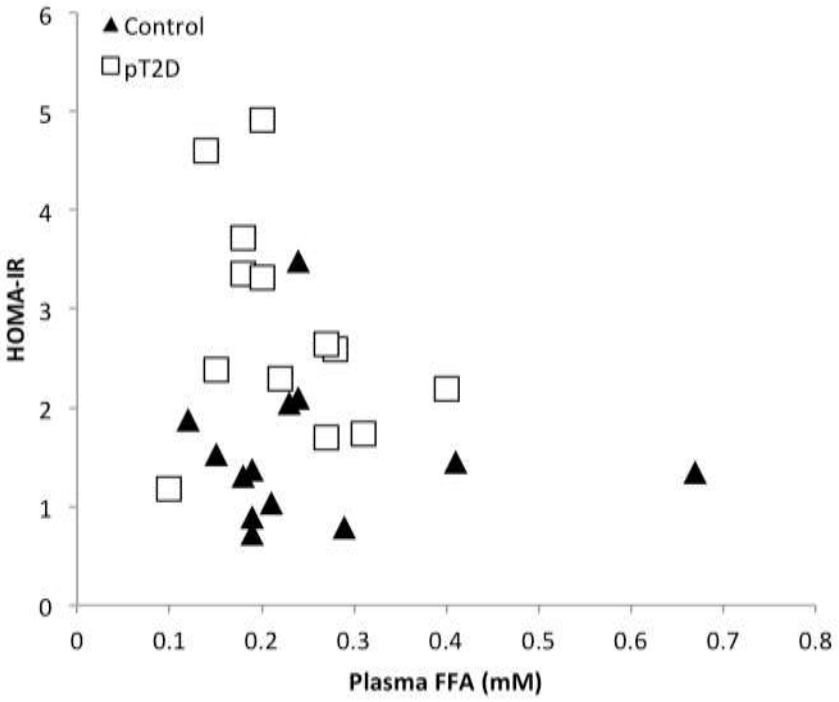


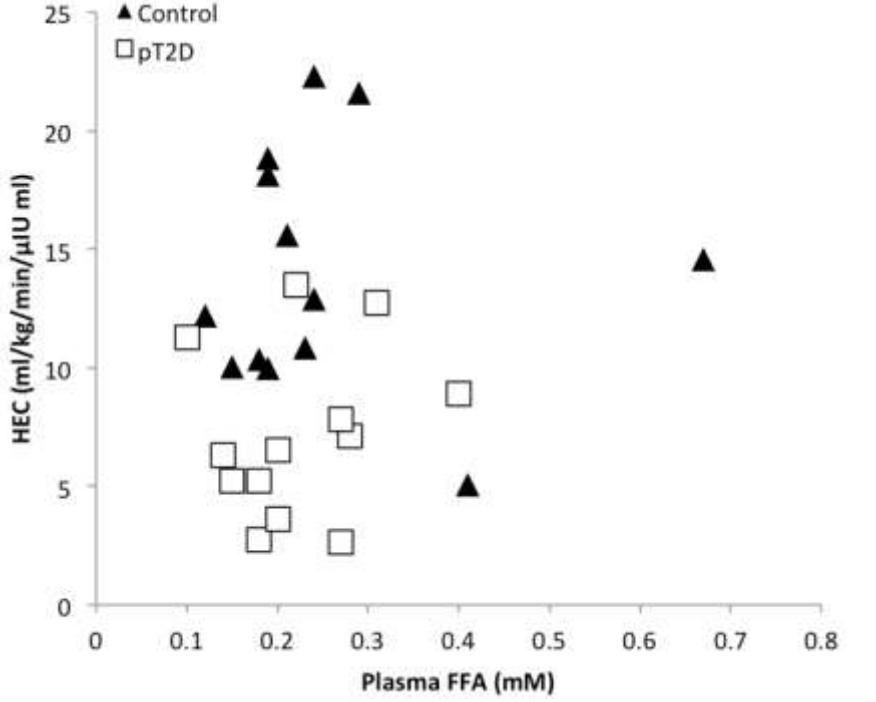
HEC = hyperinsulinemic euglycemic clamp

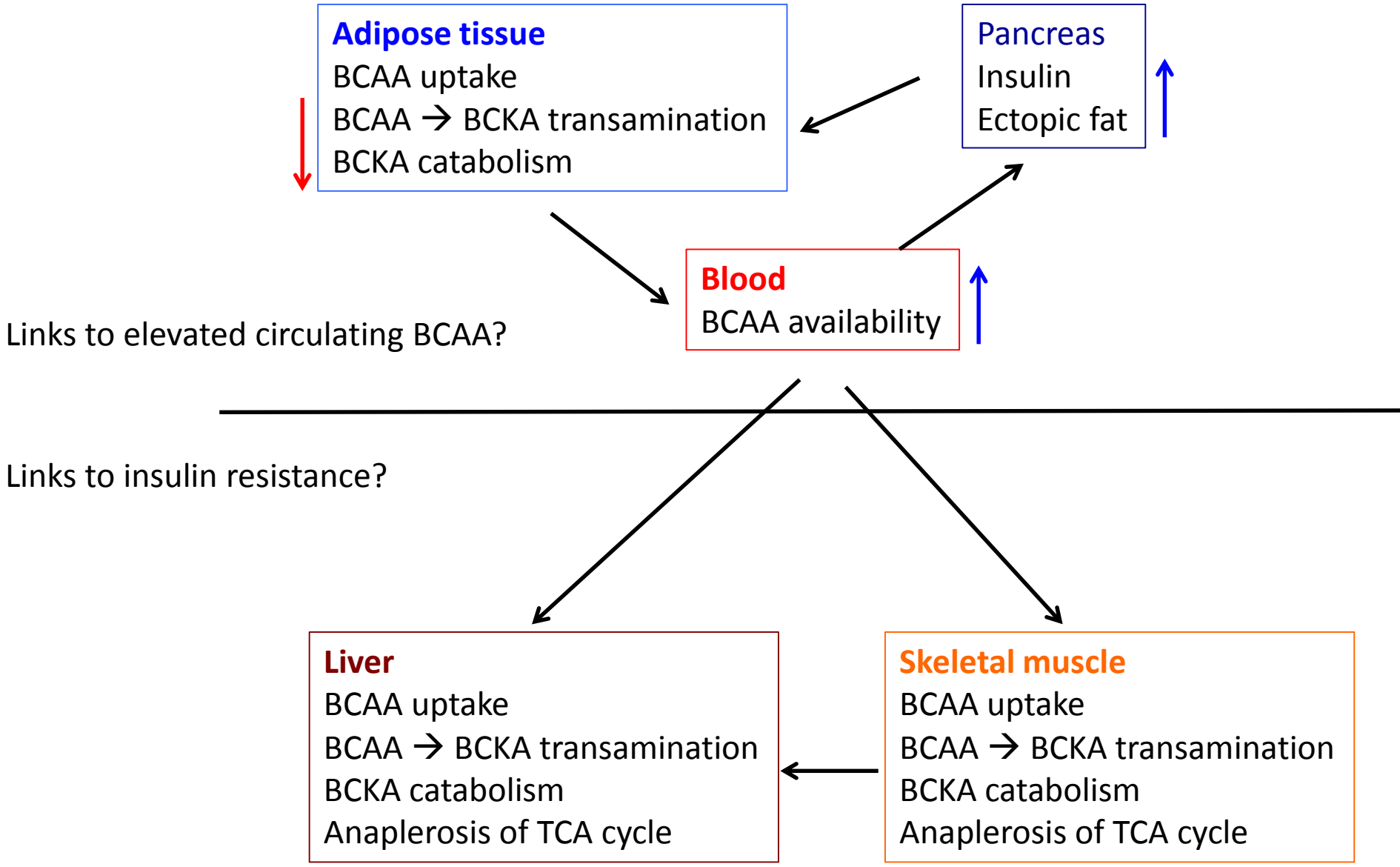
Plasma BCAAs, not FFAs, as an early marker of insulin resistance?

Newgard, 2012








Adipose tissue
BCAA uptake
BCAA → BCKA transamination
BCKA catabolism
Pancreas
Insulin
Ectopic fat
Blood
BCAA availability
Links to elevated circulating BCAA?
Links to insulin resistance?
Liver
BCAA uptake
BCAA → BCKA transamination
BCKA catabolism
Anaplerosis of TCA cycle
Skeletal muscle
BCAA uptake
BCAA → BCKA transamination
BCKA catabolism
Anaplerosis of TCA cycle

Testing if BCAA influences glucose oxidation in cell culture of myotubes.

Skeletal muscle

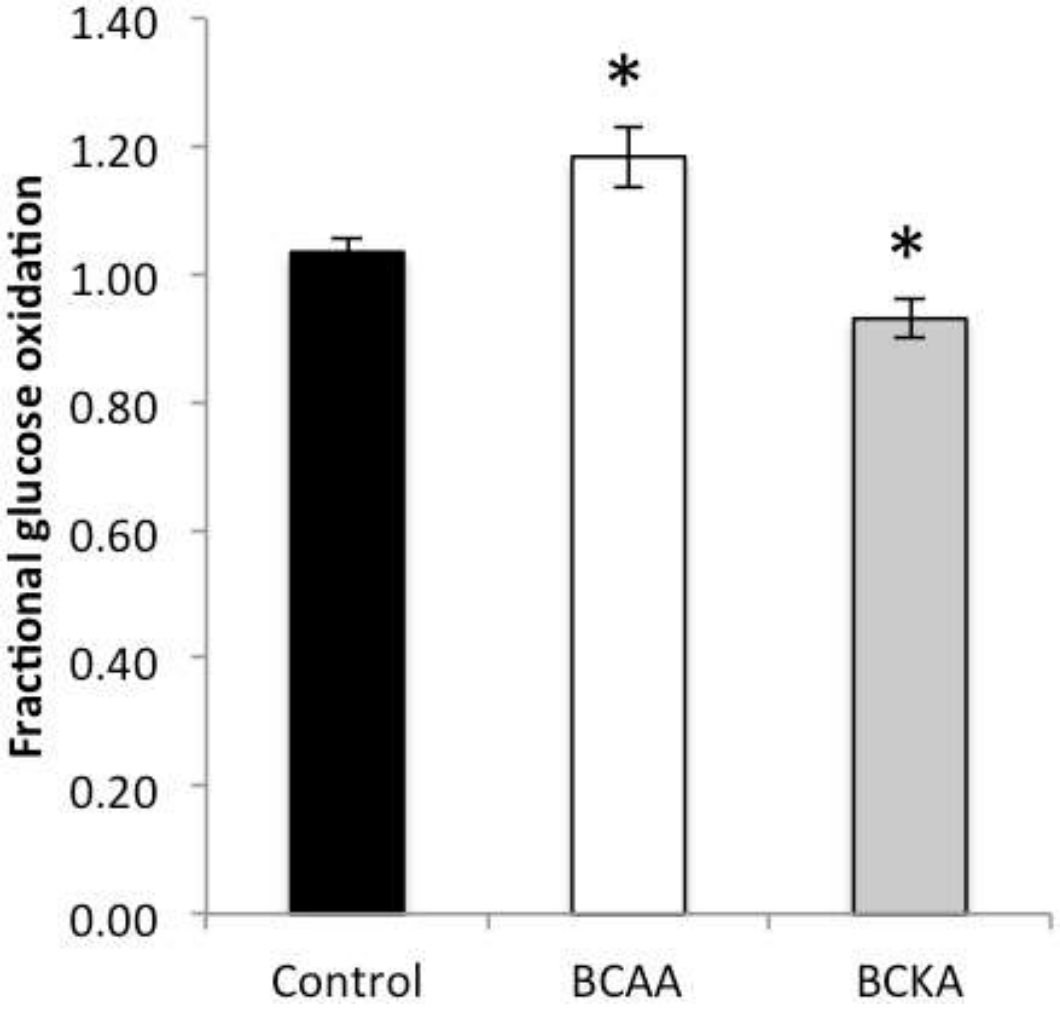
BCAA uptake

BCAA → BCKA transamination

BCKA catabolism

Anaplerosis of TCA cycle

Link between BCAAs and insulin resistance

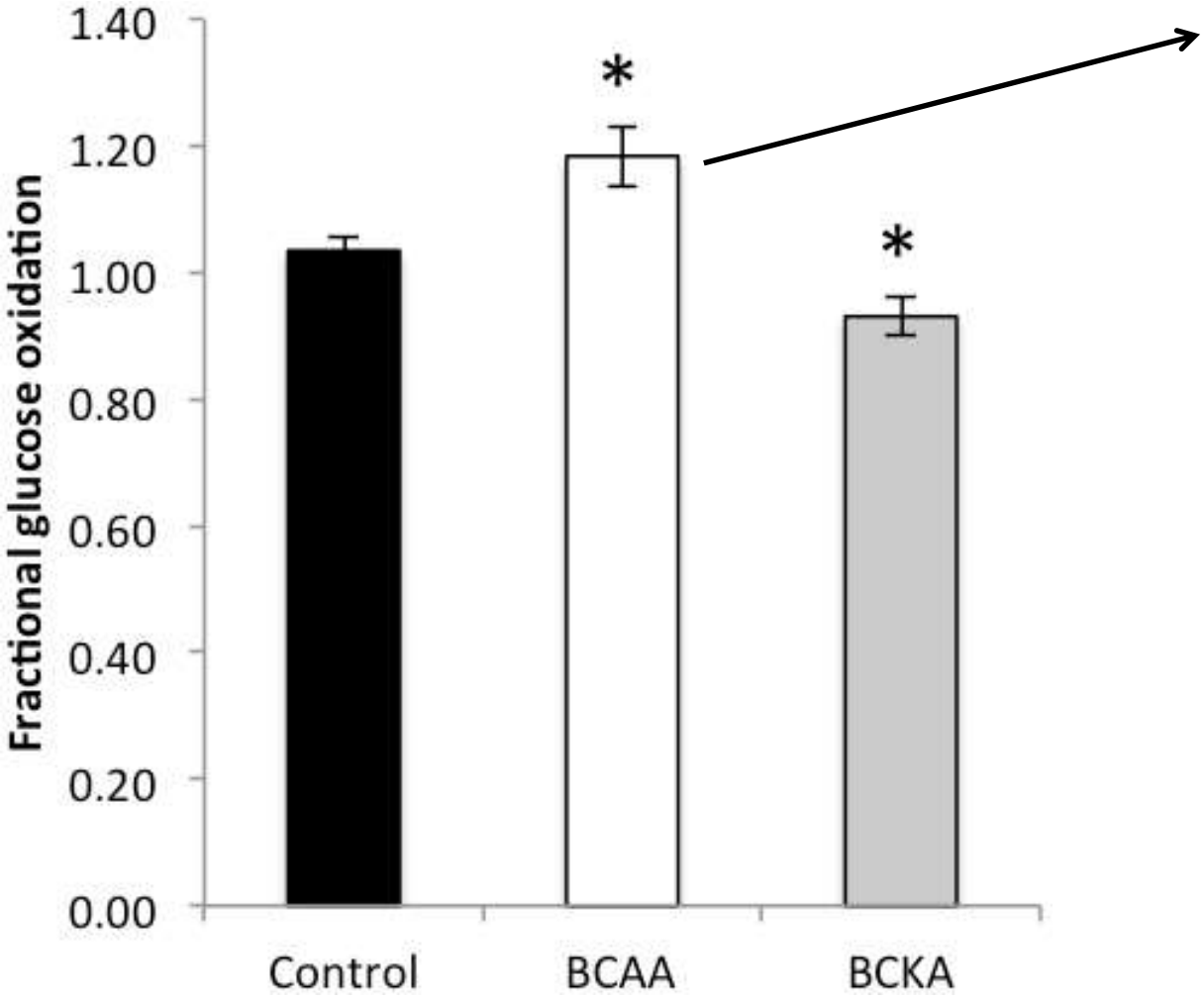


BCAA or BCKA added (10/10/10mM) to myotube culture for 24h

n=5 experiments

n=80 controls
n=40 BCAA
n=40 BCKA

Link between BCAAs and insulin resistance



↑ mTOR

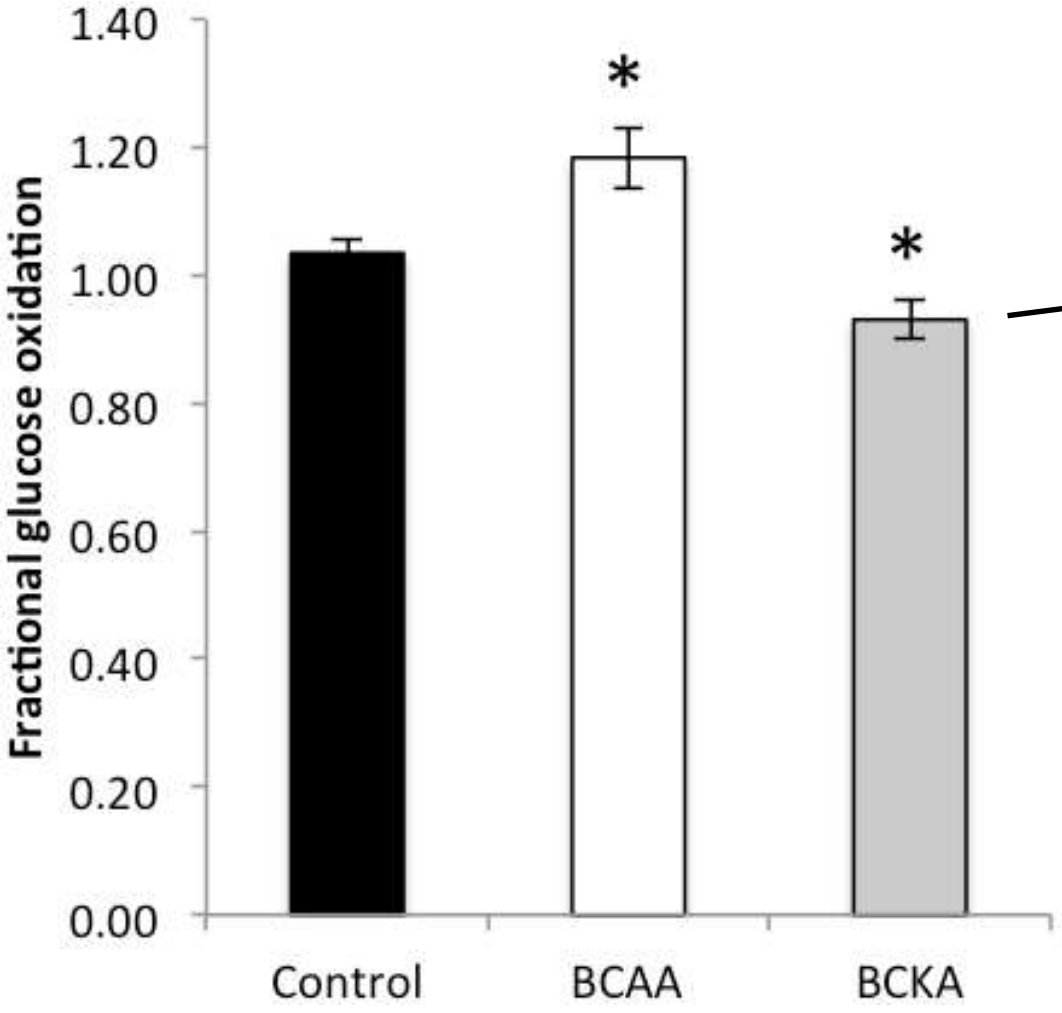
↑ Protein synthesis

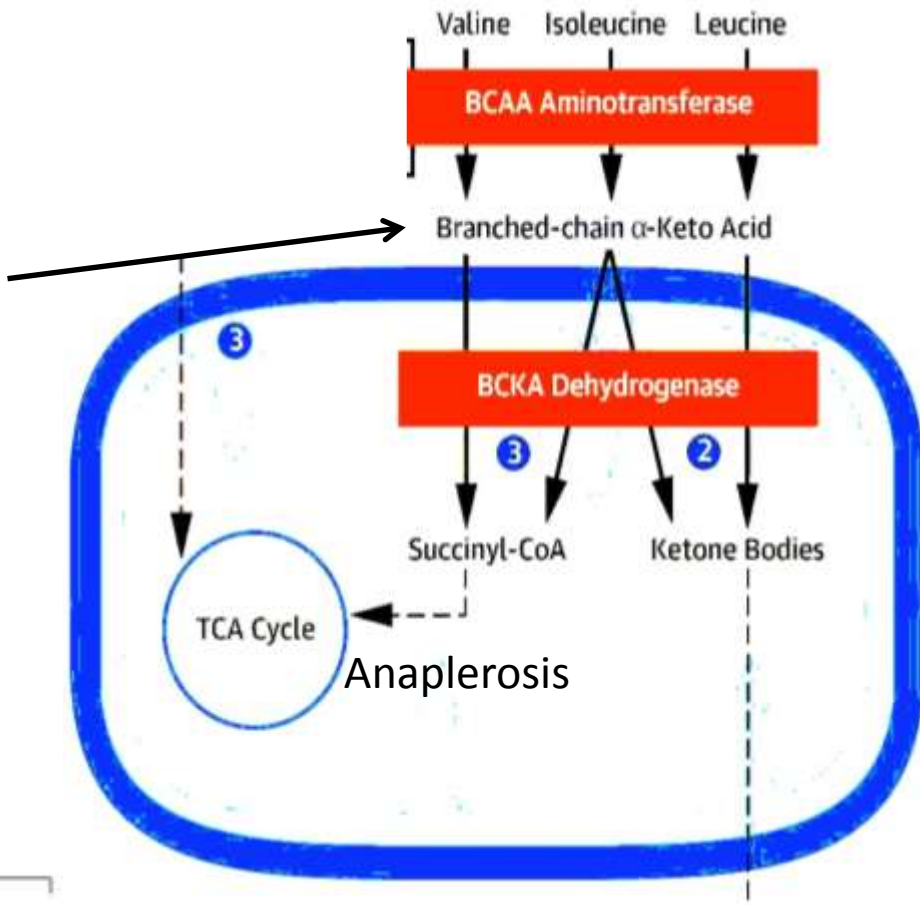
↑ GLUT3

↓ Insulin resistance

Mattic et al, 2013

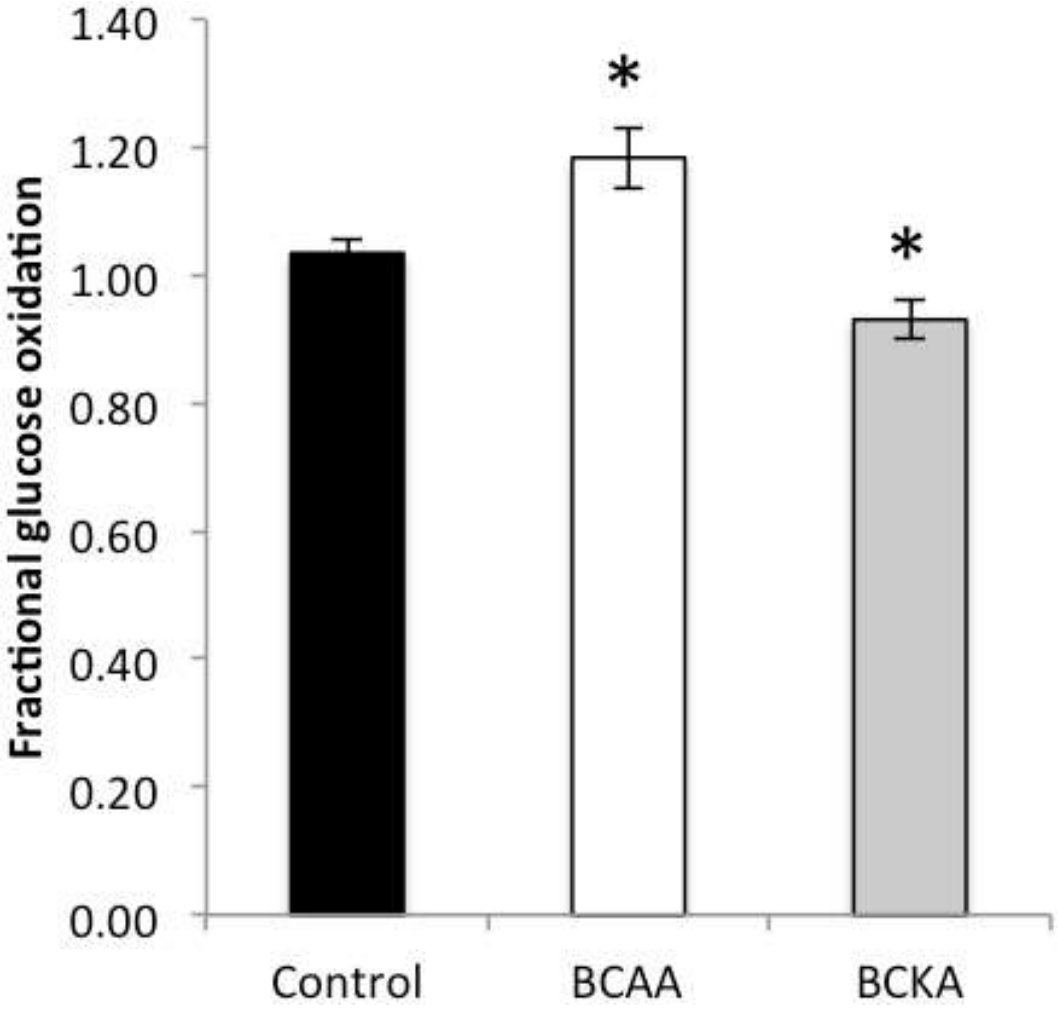
Link between BCAAs and insulin resistance



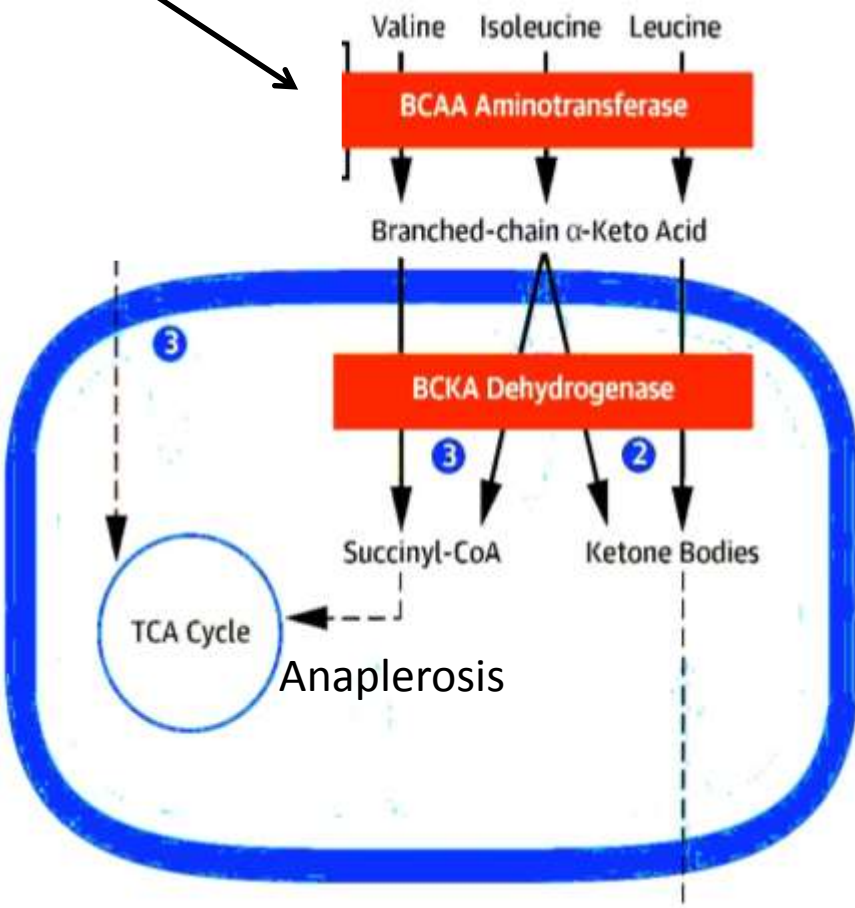


Mattic et al, 2013

Link between BCAAs and insulin resistance

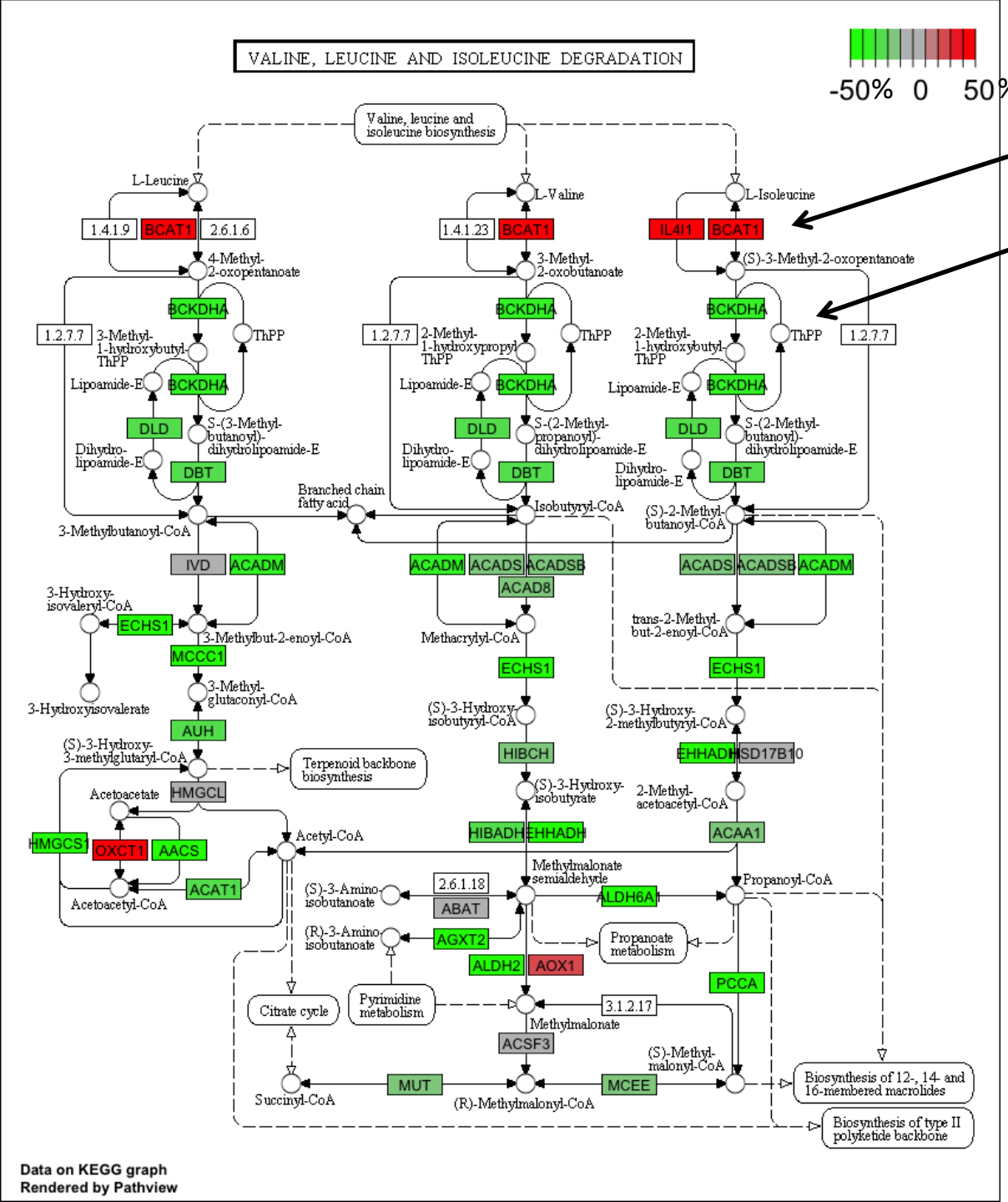


Increased in pre-diabetes?



Mattic et al, 2013

In vivo



Increased transamination

Reduced BCKA catabolism

BCKA accumulations?

Pre-diabetes:

Higher availability from plasma BCAA

Higher transamination

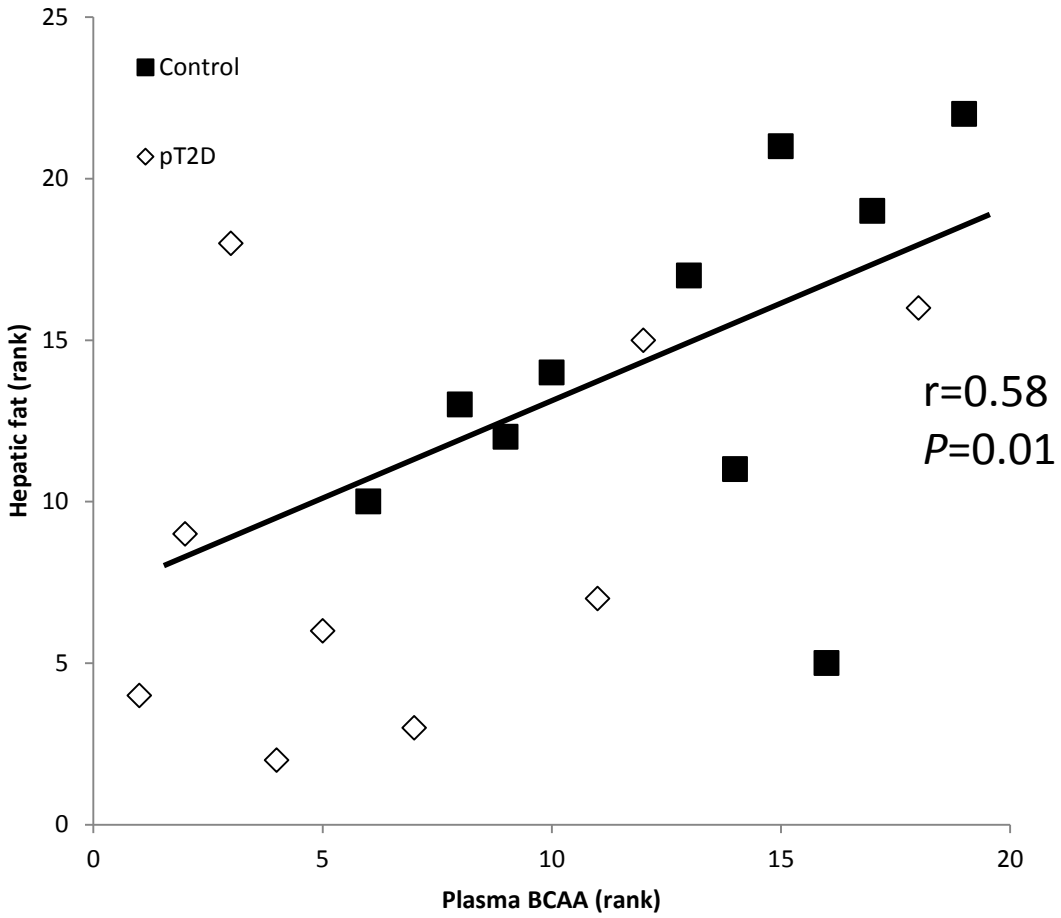
Reduced BCKA catabolism

BCKA accumulation

TCA cycle anaplerosis

Reduced glucose oxidation

Not only in SkM, also in liver?



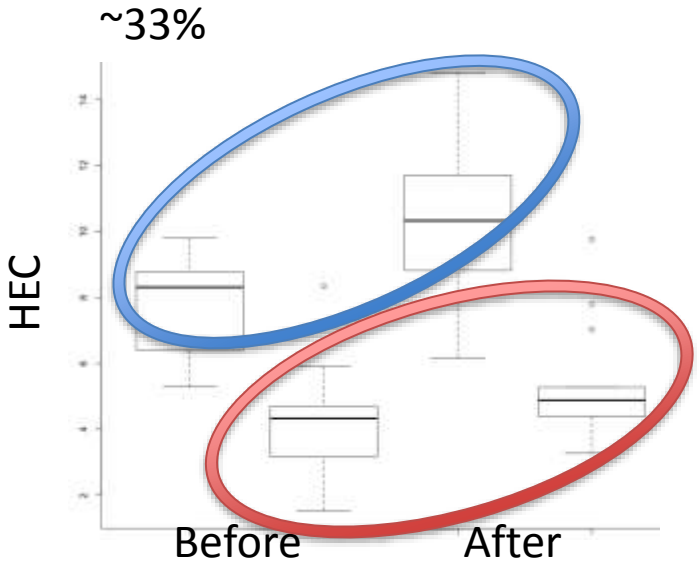
Skeletal muscle
BCKA

Liver
BCKA uptake
Anaplerosis of TCA cycle

Limited data, but consistent with non-alcoholic fatty liver disease BCKA and insulin resistance

Zhang et al 2016

12wk exercise

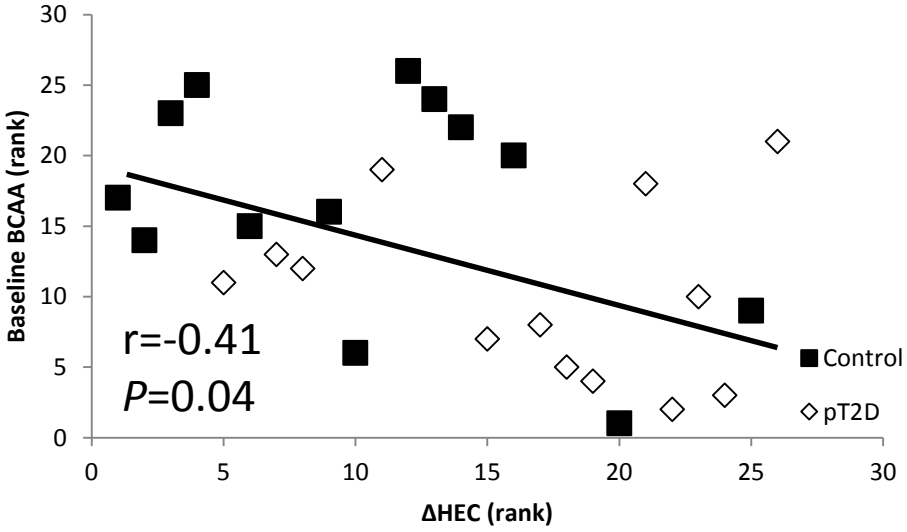


Increased insulin sensitivity by long-term exercise
Circulating BCAAs predicted change in insulin sensitivity

In line with other studies:

Are prognostic for intervention outcomes (Shah, Svetkey, et al, *Diabetologia* 55: 321, 2011)

Are prognostic for development of type 2 diabetes (Wang, et al. *Nature Med.* 17: 448, 2011; Palmer, et al. *JCEM* 100: E463, 2015)



Summary

1. **Increased plasma BCAA (not FFA) concentrations in pre-diabetes**
    1. Vicious cycle: Reduced BCAA catabolism and increased insulin
2. **BCAA interferes with glucose oxidation**
    1. Increased BCAA transamination and reduced BCKA catabolism in tissues, BCKA overflow → anaplerosis → reduced glucose oxidation?
3. **Plasma BCAA predicted the change in insulin sensitivity with 12wk exercise**

Plans for the future

Tissue measurements of BCAA and BCKA levels *in vivo*

BCKA in plasma *in vivo*

Measure enzyme activity, not only mRNA expression

In vitro studies on other types of cells (hepatocytes, adipocytes), BCAA+insulin pathway experiments ++

Gut bacteria, BCAA and exercise?

Thank you!!

PhDs/postdocs/researchers

- **Christian A. Drevon (Professor)**
- Ingrid MF Gjelstad
- Kenneth Strømmen
- Elin Blakstad
- Astrid N. Almås
- Sissel Moltu
- Fred Haugen
- Frode Norheim
- Torgrim Langleite
- Marit Hjort
- Torgeir Holen
- Kristin Eckardt
- Sindre Lee
- Shirin Pourteymour
- Kathrine Vinknes
- Thomas Olsen
- Helga Refsum

External collaborators

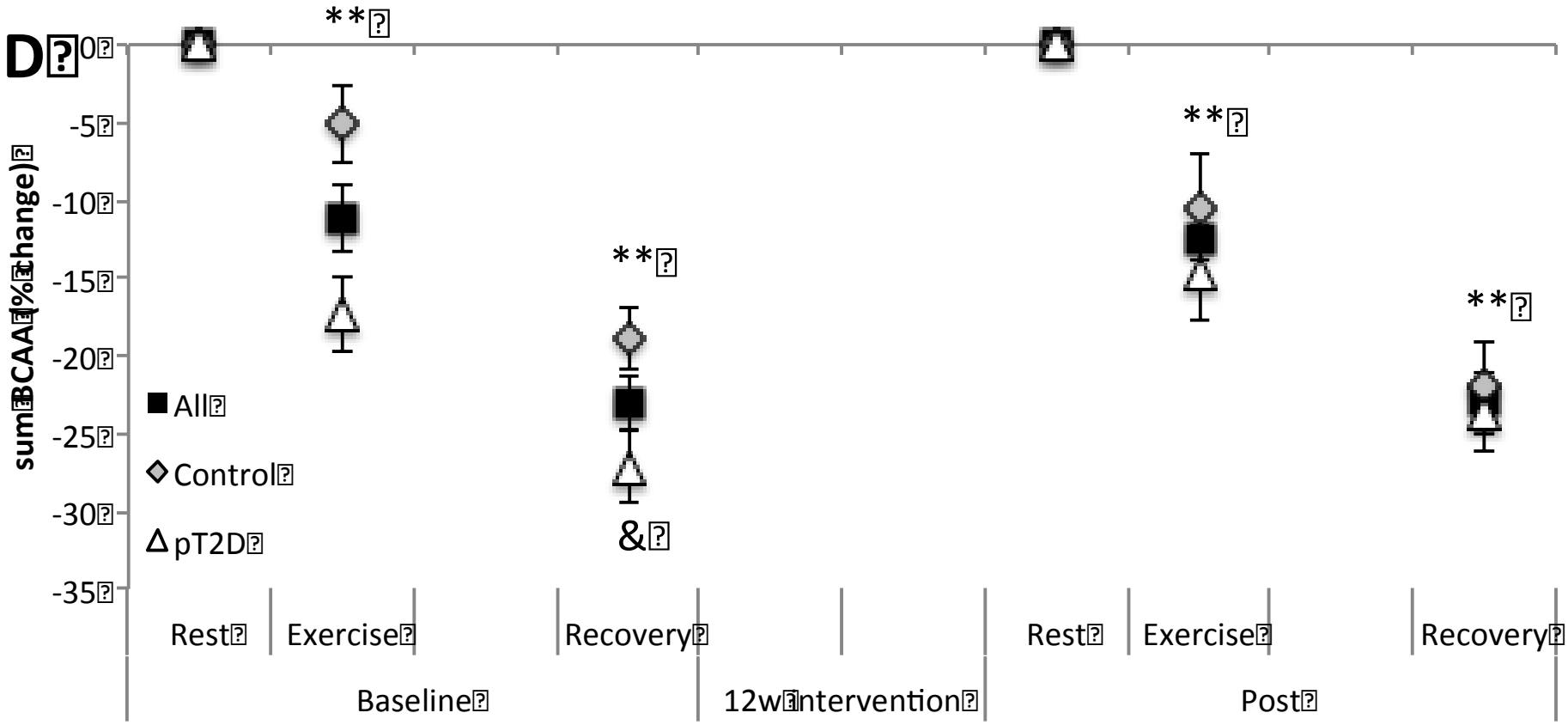
- Arild C. Rustan; fatty acid biology
- Jørgen Jensen; physical activity
- Kåre I. Birkeland/Hanne L. Gulseth
- Bernd Thiede; proteomics
- Per K. Hol et al. MRI
- Per O. Iversen, Ola D. Saugstad, Britt Nakstad, Arild Rønnestad; neonatology
- Jens P. Berg; MR metabolomics
- Thomas E. Gundersen, Vitas; lipidomics
- Willem de Vos; microbiome
- **NuGO,** B van Ommen, H Daniel, Robert Caesar
- **Food4Me**, The Gibneys et al
- **NutriTech**, B van Ommen et al
- **MyoGlu**, Birkeland et al; endocrinology
- **Lifebrain**, Kristine Walhovd/ Anders Fjell

Is increased plasma BCAA concentrations a "symptom" or "cause" of T2D?

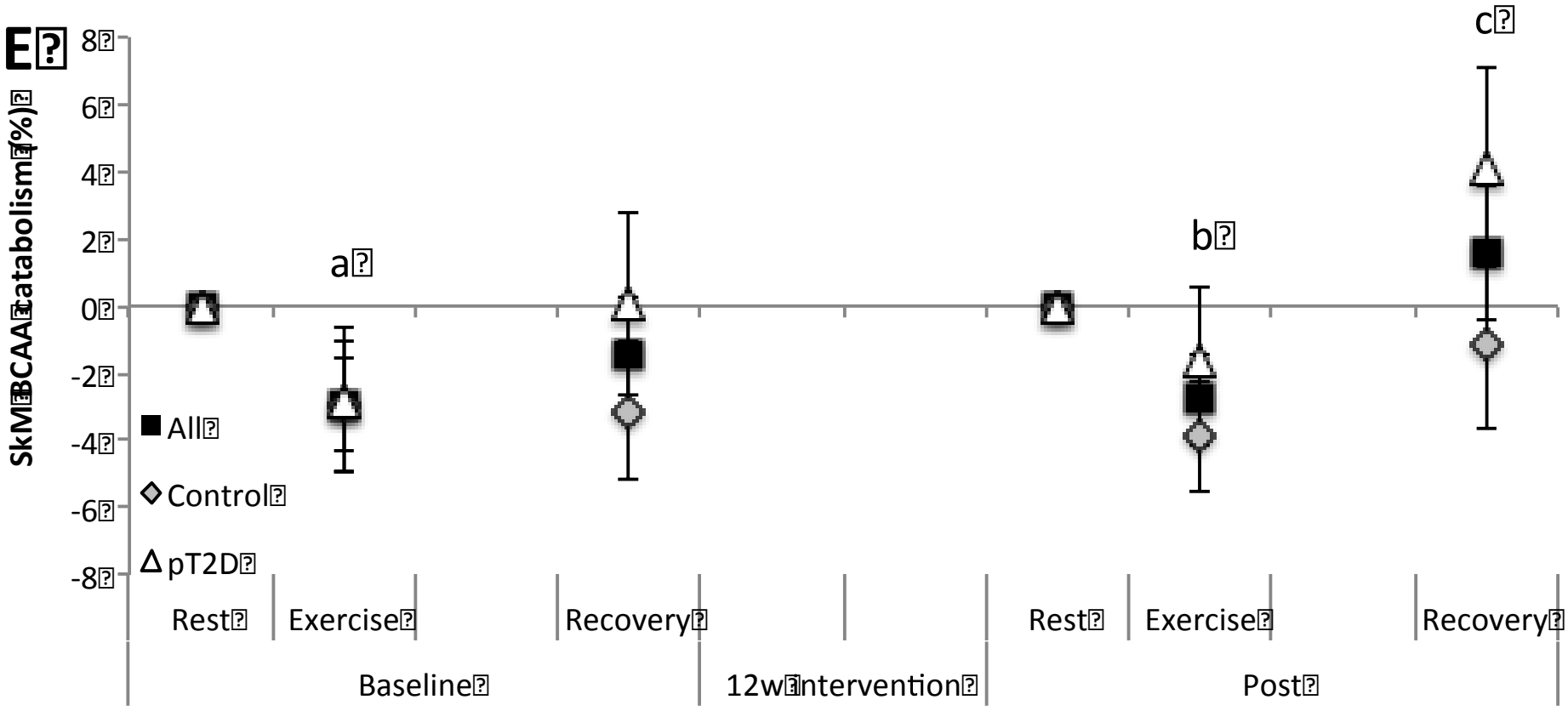
Send answer to: sindre.lee@medisin.uio.no

Extra slides…

Acute exercise



Acute exercise



Gut microbiota

Essential amino acids

(must check FFQs..)

Genetics

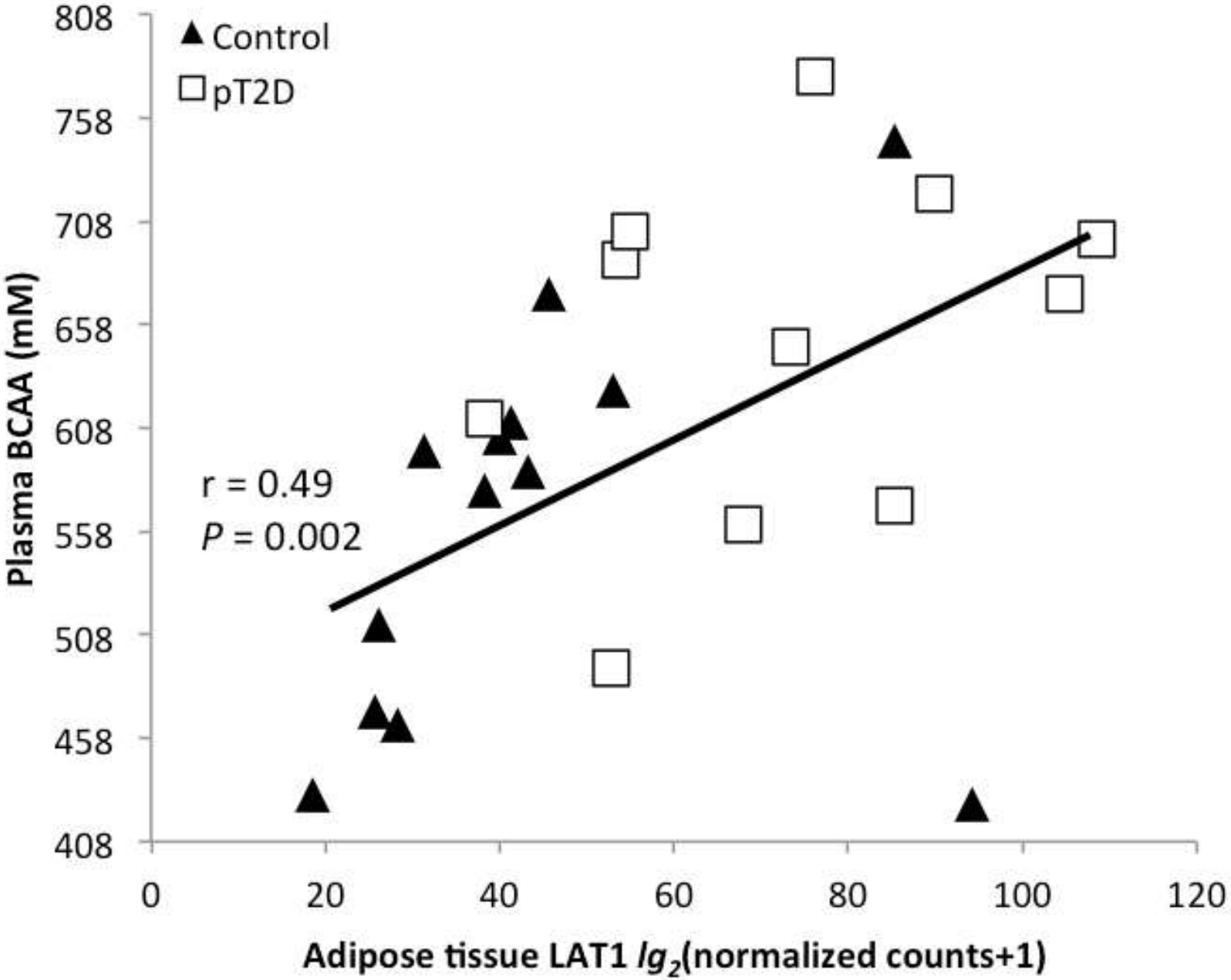
Adjustment for cofounders?

Underpowered study….

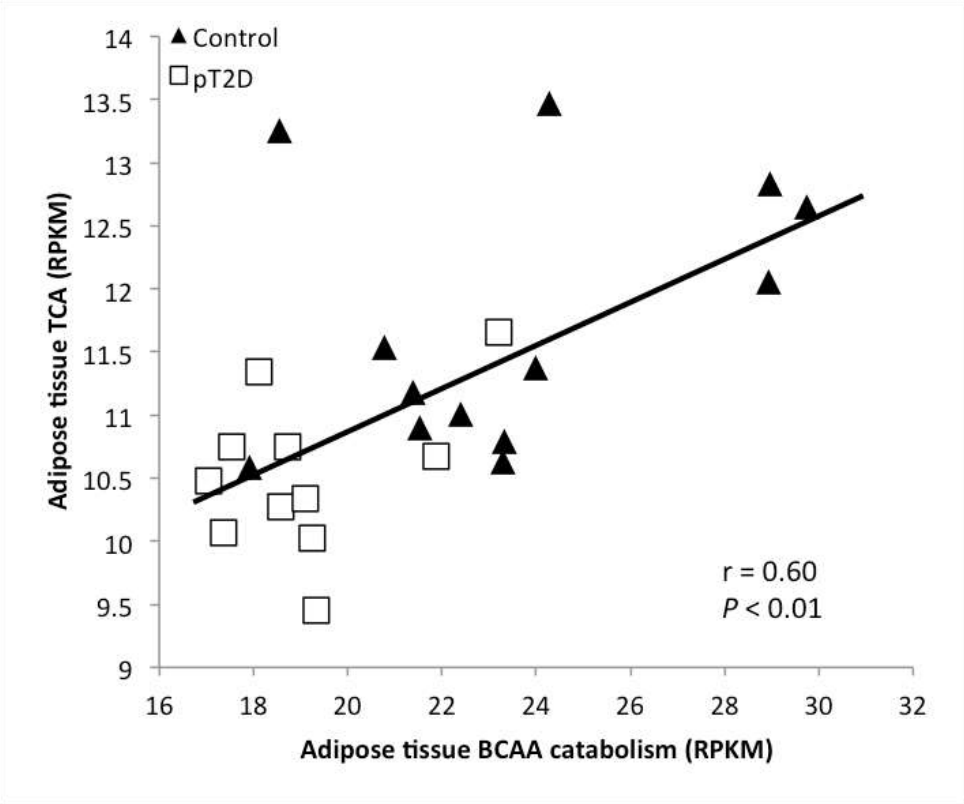
Insulin, glucose, ectopic fat ++

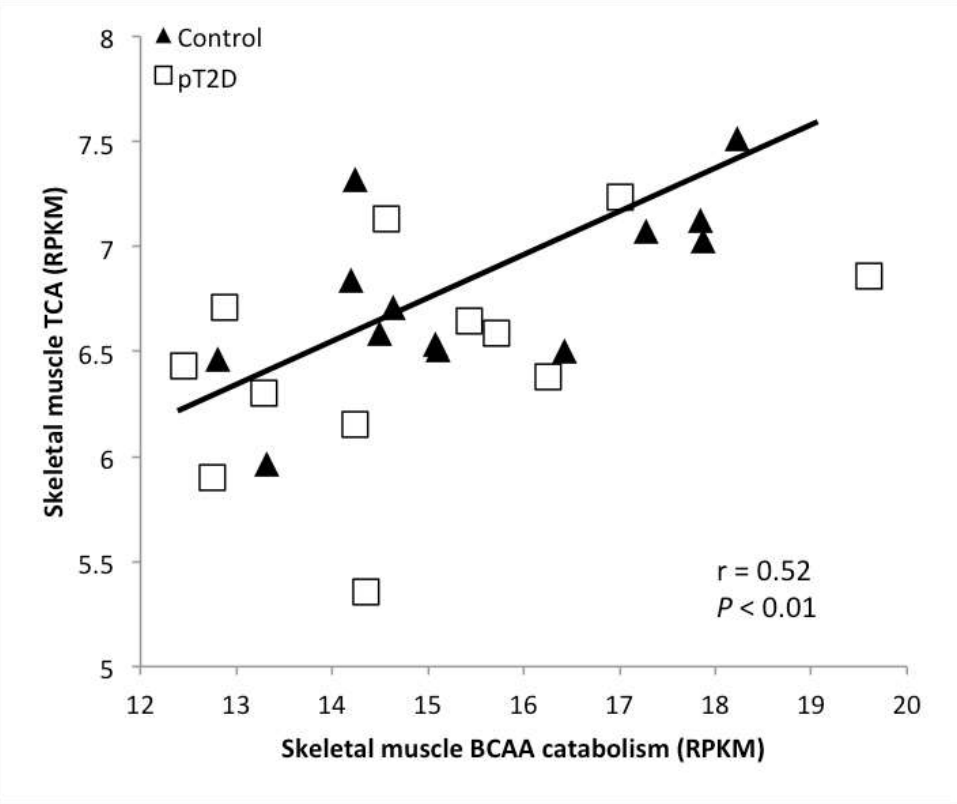
Newgard et al 2012
Sunny et al 2016
Gougeon et al 2008

Why elevated plasma BCAA concentrations?



BCAA catabolism correlates with TCA cycle mRNA





BCAA responses to exercise