Normalizing Energy Expenditure in Mice

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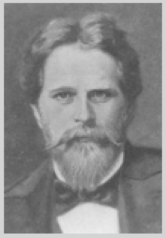
Late 1700s — Antoine Lavoisier and Pierre-Simone Laplace: body mass is a major determinant of EE

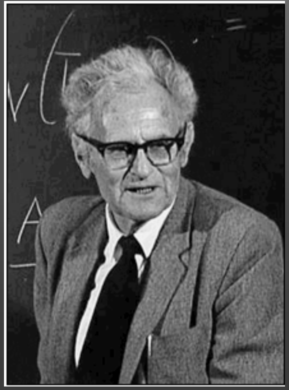
Thus, "normalization" is needed to:

- Evaluate the effect of a variable on EE without confounding by differences in body size
- Determine whether a variable changes the per-kg EE value of body mass or a body mass compartment
- EE (cal/min) = VO_2 (ml/min) X 5

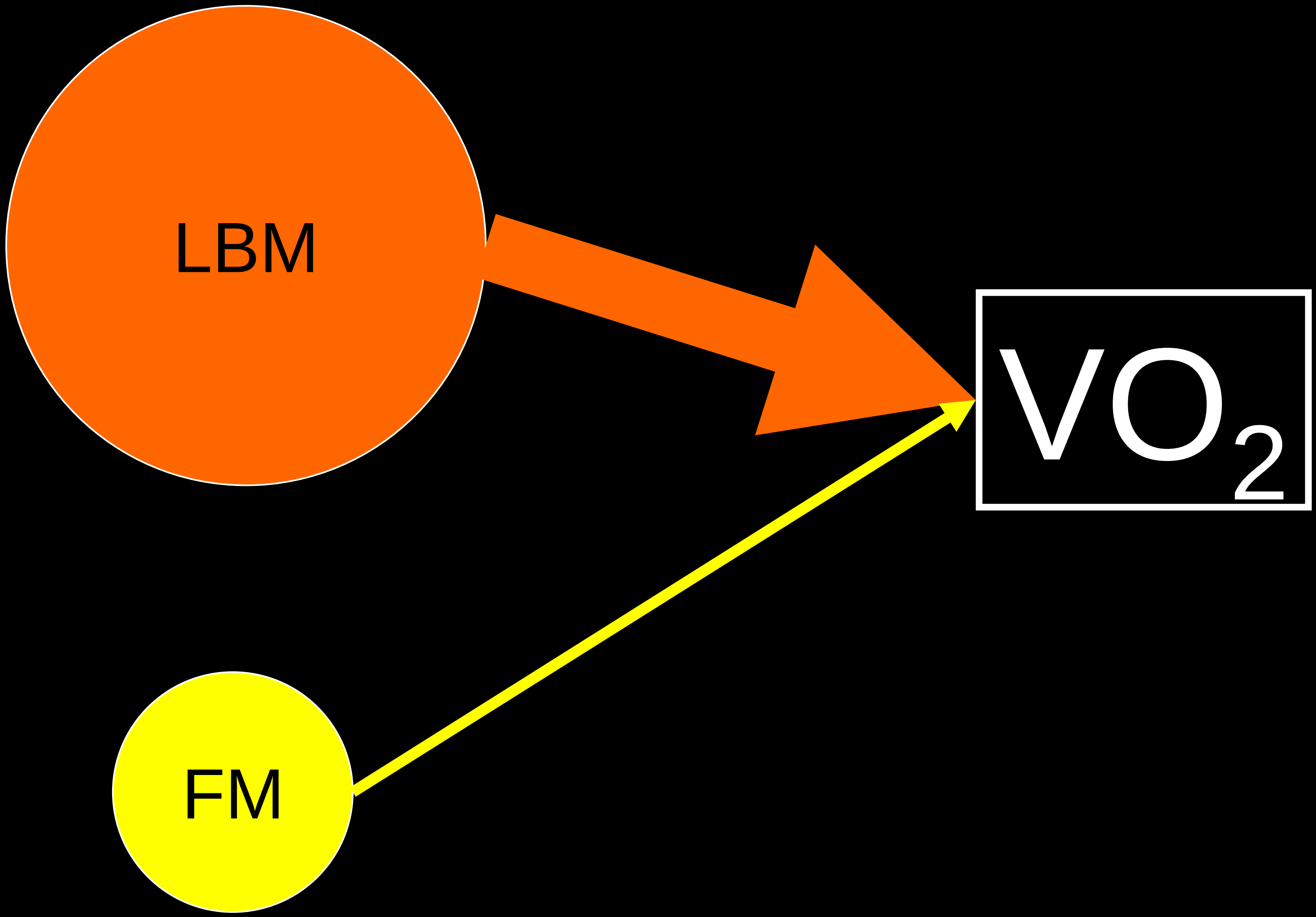
Common methods of normalization

Method		Assumption
1 Anonymous	$\frac{VO_2}{\text{Body Mass}}$	$VO_2 = C \text{ Body mass}^1$
2 Rubner (1888)	$\frac{VO_2}{\text{Body Mass}^{.67}}$	$VO_2 = C \text{ Body mass}^{.67}$
3 Kleiber (1932)*	$\frac{VO_2}{\text{Body Mass}^{.75}}$	$VO_2 = C \text{ Body mass}^{.75}$





Standard model of the major determinant of VO_2



And so, with the advent of small animal body composition technology….

$$VO_2norm = \frac{VO_2}{LBM}$$

Assumes

VO_2 = C x LBM

AND

$$VO_2 = C \times LBM$$

FM

But what about me?

Question

Do the commonly used normalization methods remove the effect of varying body size from VO_2 in mice?

Approach

Compare four different normalization methods using data from 142 mice (C57B6) evaluated for VO_2 and body composition in Seattle's MMPC

- 66 wildtypes (25 female, 41 male)
- 76 mutants (28 female, 48 male; none were models of monogenic obesity)
- 63 were tested twice (once on chow, then on HFD)
- Total number of evaluations = 205.

Some details

- All mice were acclimated to and then studied in calorimeters for 36 to 48 hours

- Analyzed data from second light cycle and second dark cycle

Dependent variables:

1) Minimum VO_2 recorded during light cycle

2) Average VO_2 computed over the 24 hours encompassing the light and dark cycles

Yellow = HFD

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Yellow = HFD

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TIFF (Uncompressed) decompressor
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Yellow = HFD; $p < .0001$

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TIFF (Uncompressed) decompressor
are needed to see this picture.

Yellow = HFD; $p = .56$

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Yellow = HFD; p = .2

Yellow = HFD; p = .02

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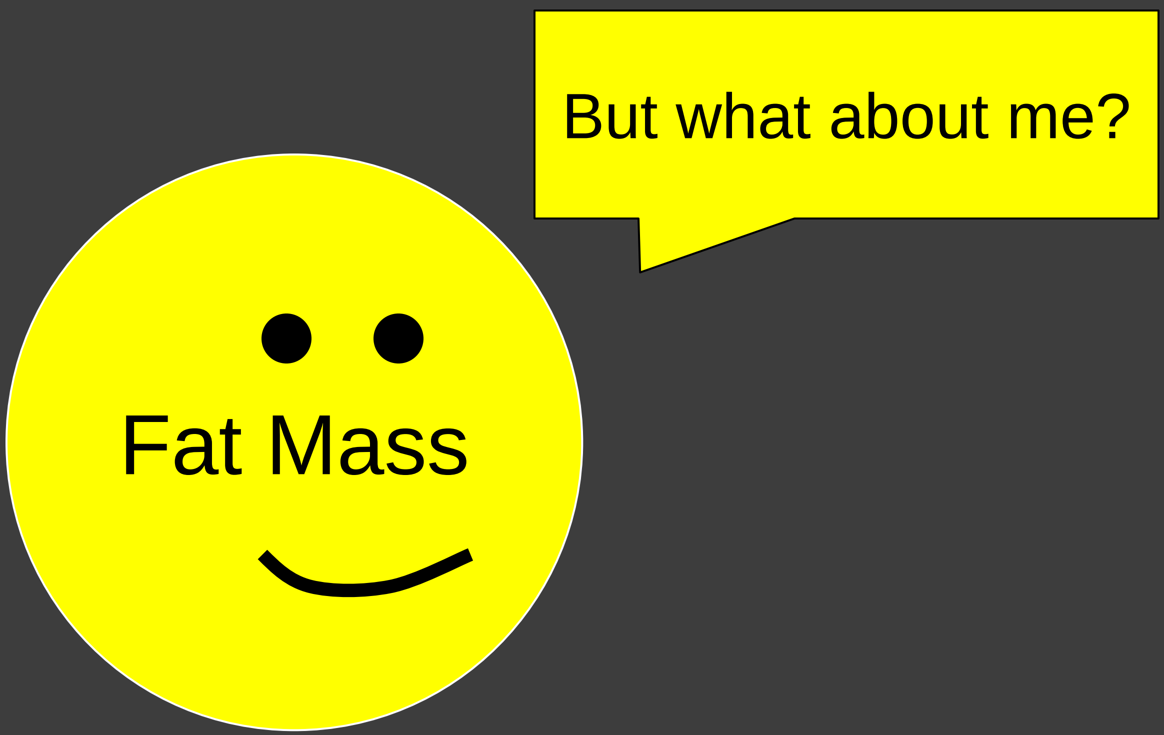
SUMMARY 1

Commonly used normalization methods

- Do not fully remove variation in body mass from the normalized VO_2 value

- Implies that other factors are important and that other approaches to normalization are needed

One thing ignored by commonly used normalization methods:



Wildtype Transgenic

> 5-Fold difference in mean FM

16

13

12

QuickTime™ and a
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are needed to see this picture.

8

6

5

4

3

QUESTION

Is fat mass an important determinant of VO_2?

APPROACH

- Assess whether fat mass is associated with VO_2 *independently* of lean body mass and other important predictors
- Used GEE to handle the 63 repeated measurements

Significant predictors of VO_2

	Significance of predictor	
	Min VO_2	Ave VO_2
FM	.000	.000
LBM	.000	.000
Diet	.000	.000
Gender	.000	.01
Genotype	.004	.02
Activity		.000

QuickTime™ and a
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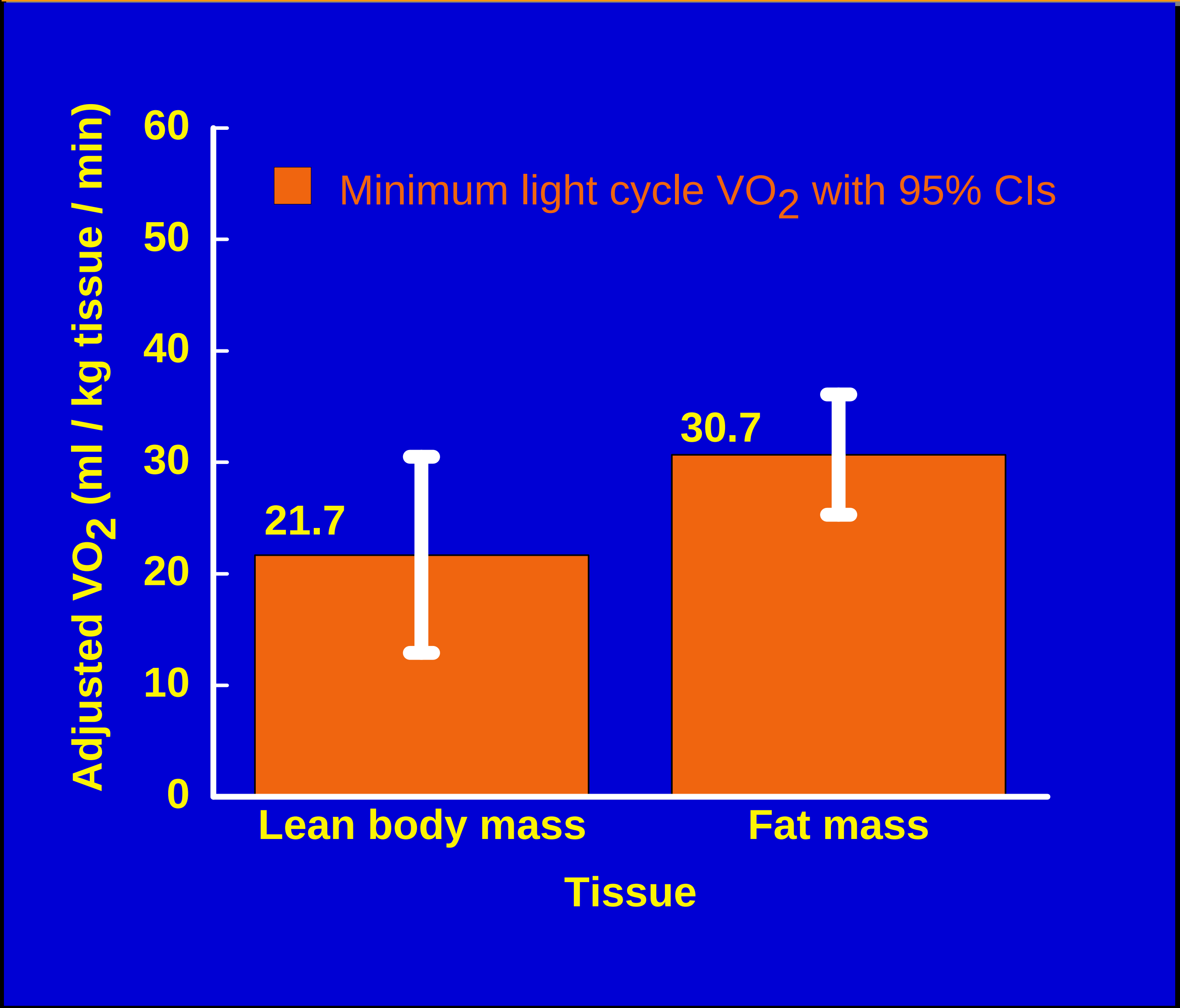
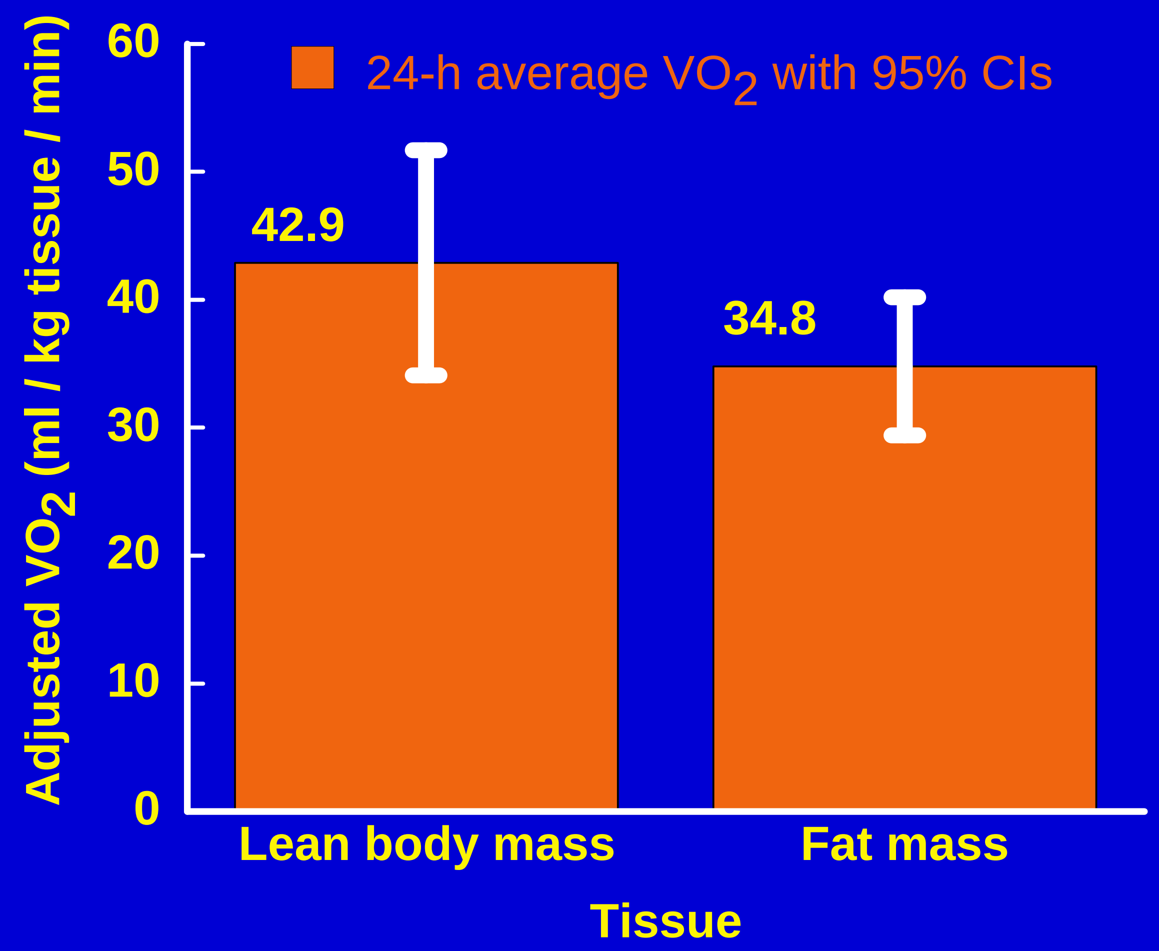
Estimated contributions of fat and lean body mass to minimum VO_2



Model includes:

- Diet
- Gender
- Genotype

Estimated contributions of fat and lean body mass to average VO_2



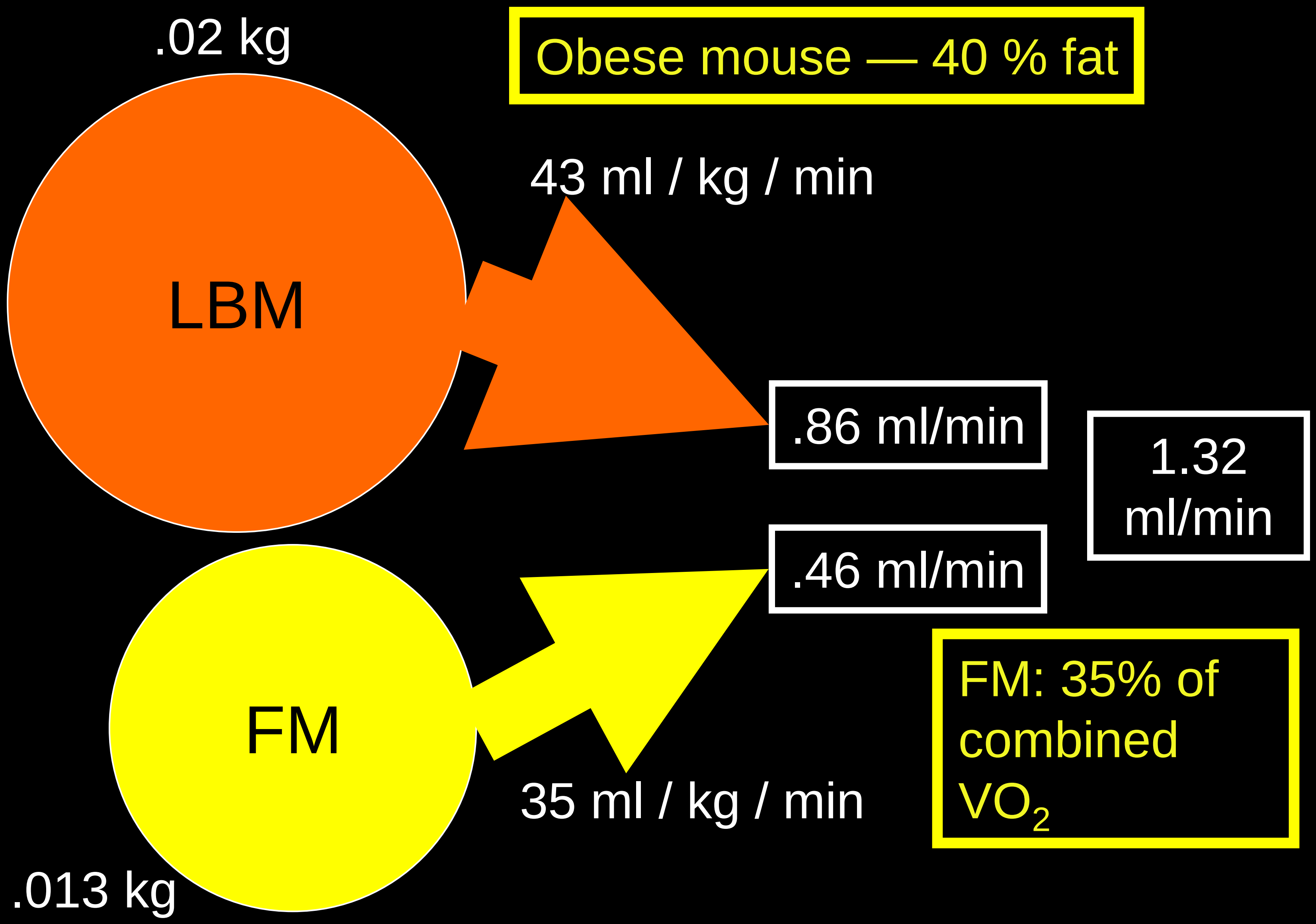
Model includes:

- Diet
- Gender
- Genotype
- Activity

The Point:

FM <u>is associated with</u> a substantial per-kg oxygen cost

Revised model applied to 24-h average VO_2



Implications for normalizing VO_2 in mice

Data analysis:

• Should *include* multiple regression or GEE to adjust VO_2 for FM, LBM and the primary independent variables *if sample sizes permit (ANCOVA)*

• But, since the regression-based oxygen costs assigned to FM and LBM are comparable, you can instead adjust for total BM and the primary I.V.s if sample sizes are modest

Example for a very typical study

• Use ANCOVA to evaluate the effects of two independent variables adjusting for BM as a covariate

• Estimates mean (95% CI) VO_2 as a function of each independent variable at the overall mean BM — variation in BM is removed from the VO_2 group means!

• Divide adjusted estimates by overall mean BM to derive a mass-specific normalization

Example using a PKA study

Count

		Genotype		
		Wildtype	Transgenic	Total
Diet	Chow	8	8	16
	High fat	8	6	14
	Total	16	14	30

Example using a PKA study

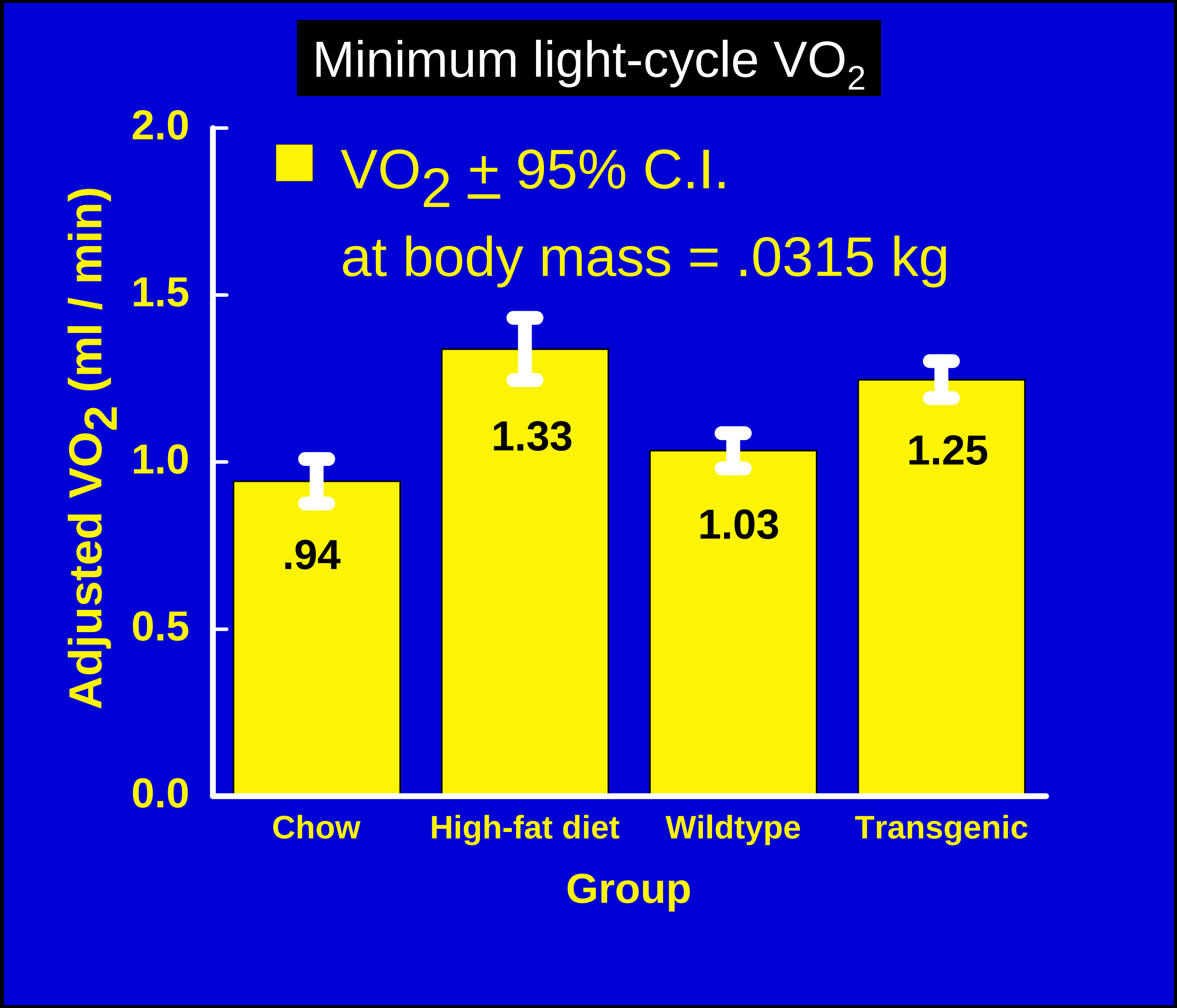
Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	.748	.2169	.323	1.173	11.912	1	.001
bodymasskg	22.131	6.0873	10.200	34.061	13.217	1	.000
[diet=.00]	-.395	.0739	-.540	-.250	28.583	1	.000
[diet=1.00]	0[a]	.	.	.	.	.	.
[genotype=.00]	-.213	.0411	-.294	-.133	26.882	1	.000
[genotype=1.00]	0[a]	.	.	.	.	.	.
(Scale)	1						

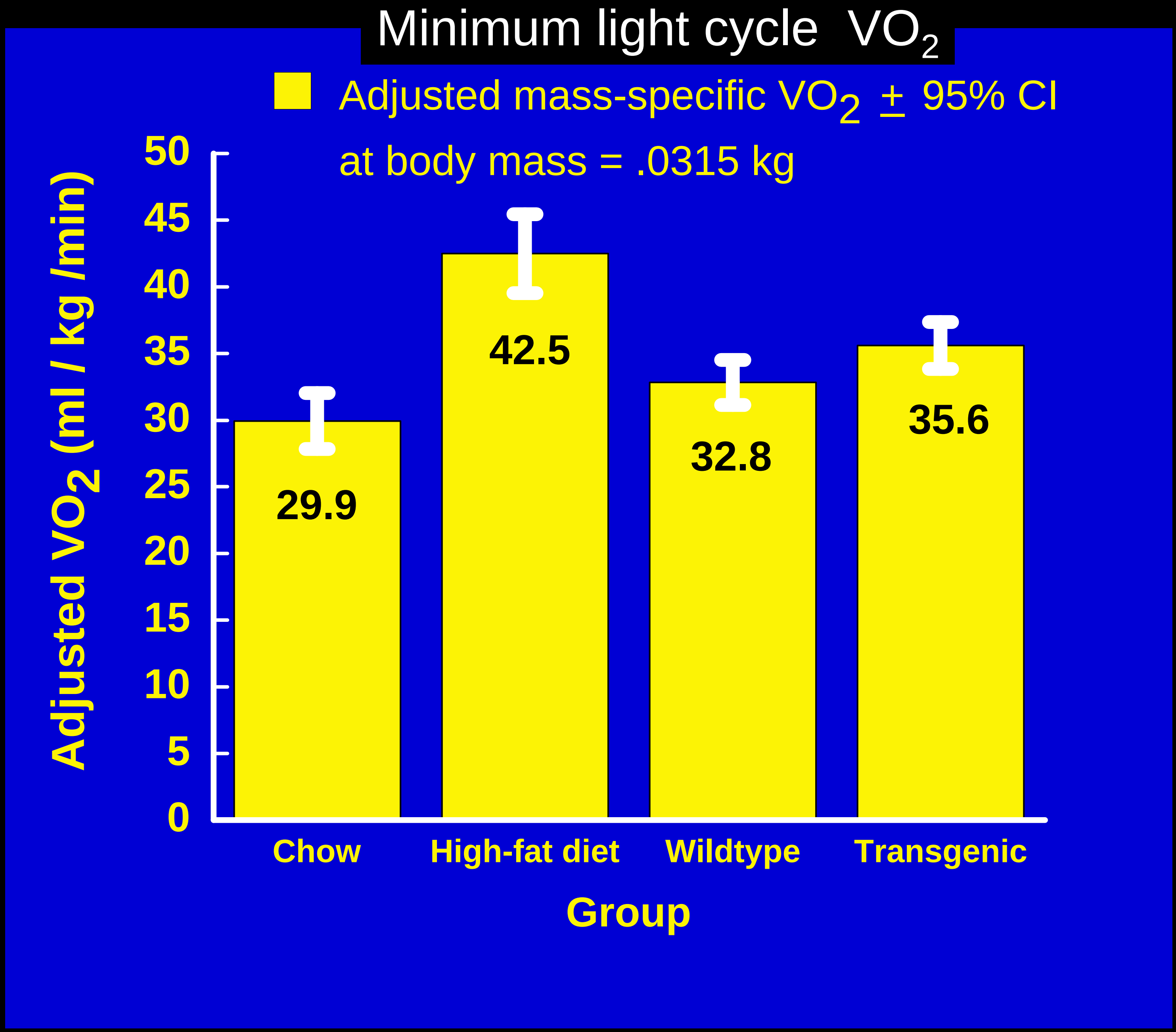
Dependent Variable: Minimum light cycle VO2 (ml/min)
Model: (Intercept), bodymasskg, diet, genotype

a. Set to zero because this parameter is redundant.

Here, the per-kg VO_2 estimate of body mass is 22.13 ml/kg/min…



Minimum light-cycle VO_2
VO_2 ± 95% C.I.
at body mass = .0315 kg
Adjusted VO_2 (ml / min)
2.0
1.5
1.0
0.5
0.0
.94
1.33
1.03
1.25
Chow
High-fat diet
Wildtype
Transgenic
Group



Minimum light cycle VO2
Adjusted mass-specific VO2 ± 95% CI
at body mass = .0315 kg
Adjusted VO2 (ml / kg /min)
50
45
40
35
30
25
20
15
10
5
0
29.9
42.5
32.8
35.6
Chow
High-fat diet
Wildtype
Transgenic
Group

Example from a study with larger sample sizes

Count

		Genotype		
		Wildtype	Transgenic	Total
Diet	Chow	20	20	40
	High fat	10	10	20
	Total	30	30	60

Example from a study with larger sample sizes

Dependent variable: 24-h average VO_2

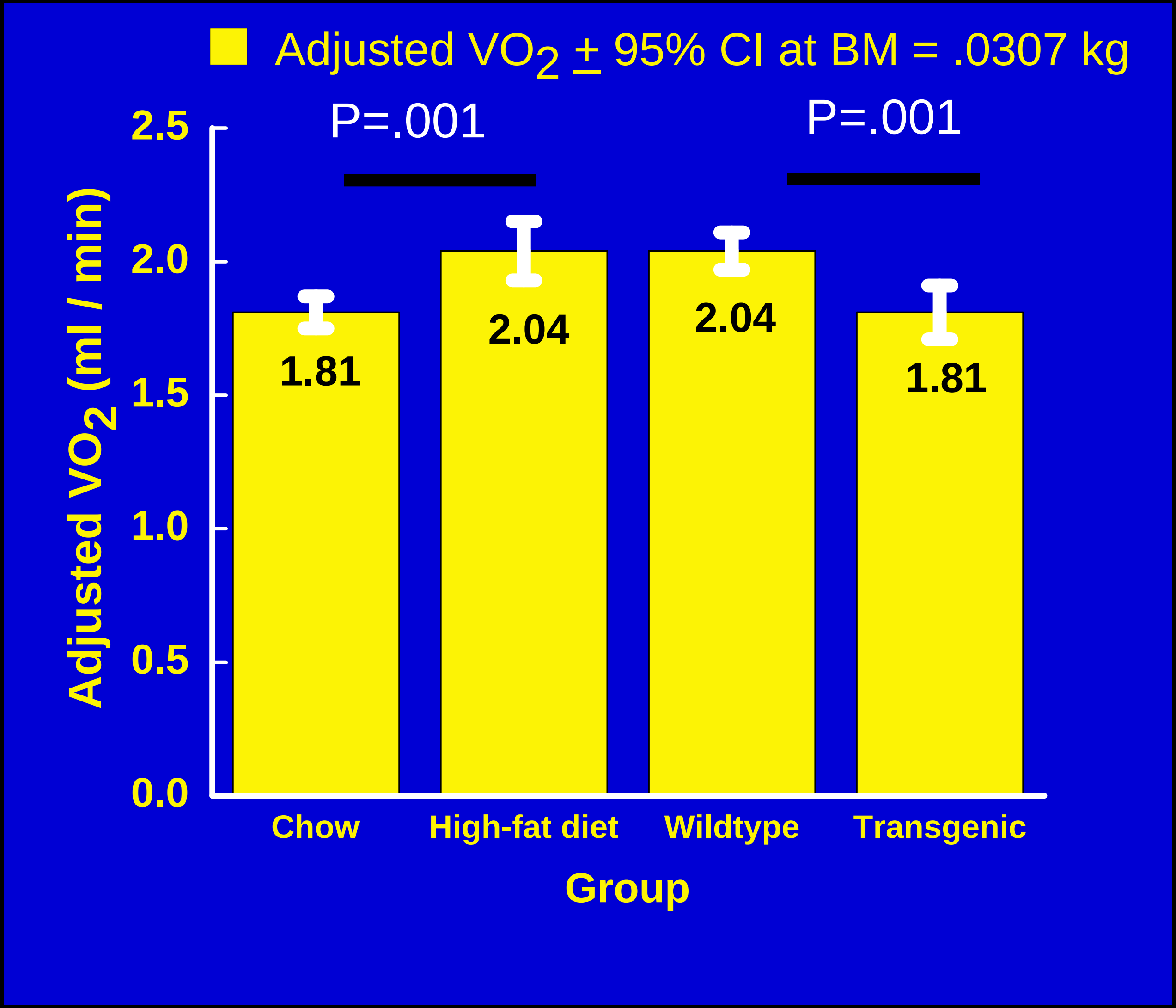
Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	.774	.1680	.445	1.104	21.251	1	.000
leankg	38.378	11.2745	16.281	60.476	11.587	1	.001
fatkg	38.650	6.3139	26.275	51.025	37.472	1	.000
[diet=.00]	-.230	.0693	-.365	-.094	10.976	1	.001
[diet=1.00]	0[a]	.	.	.	.	.	.
[genotype=.00]	.234	.0712	.094	.373	10.794	1	.001
[genotype=1.00]	0[a]	.	.	.	.	.	.
(Scale)	1						

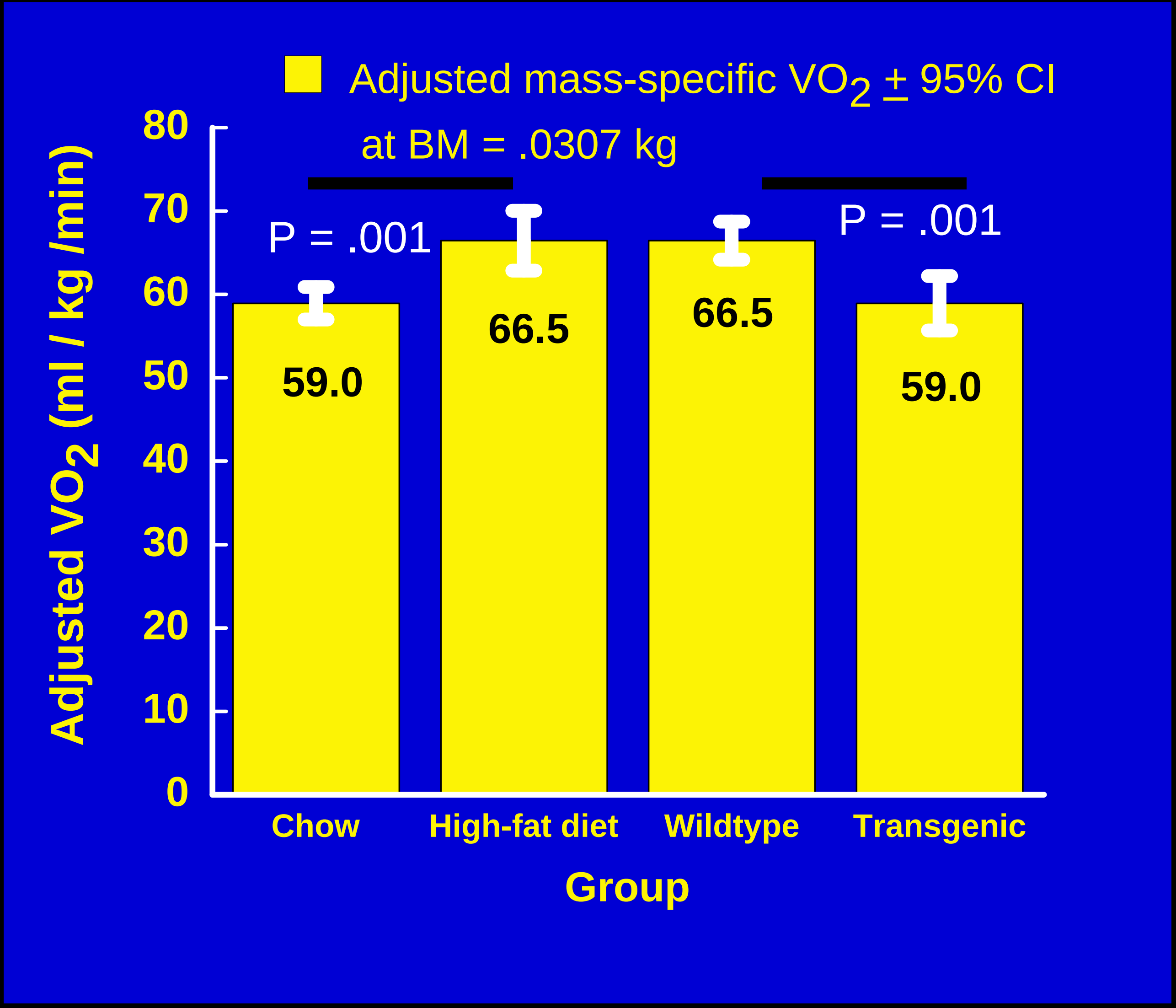
Dependent Variable: 24-h mean VO2 (ml/min)
Model: (Intercept), leankg, fatkg, diet, genotype

Here, the LBM and FM per-kg VO_2 estimates are almost identical at about 38.5 ml/kg/min…

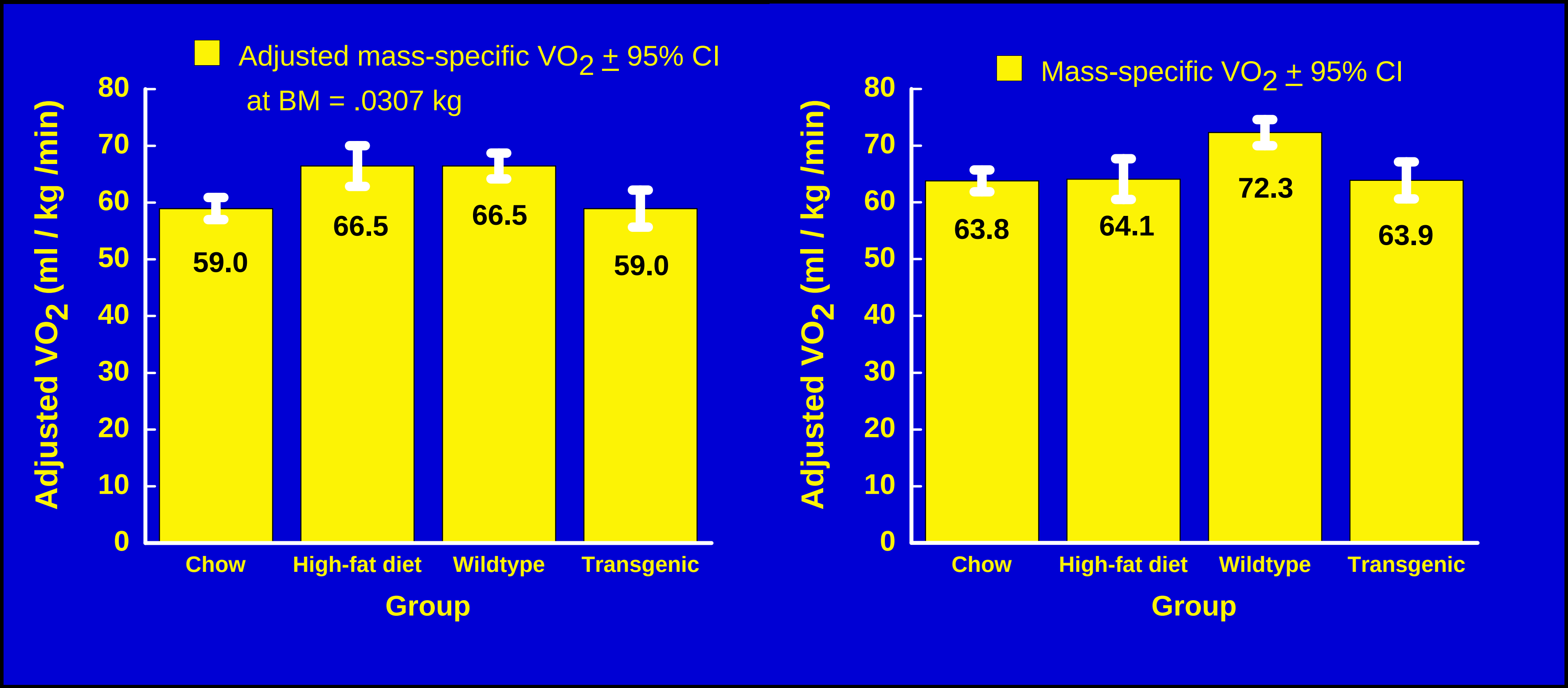
24-h average VO2



Example using a model with larger sample sizes



What if we had just compared mass-specific VO_2 values using the historical method ?



Diet and genotype both significant

Diet effect not significant

SUMMARY 2

ANCOVA

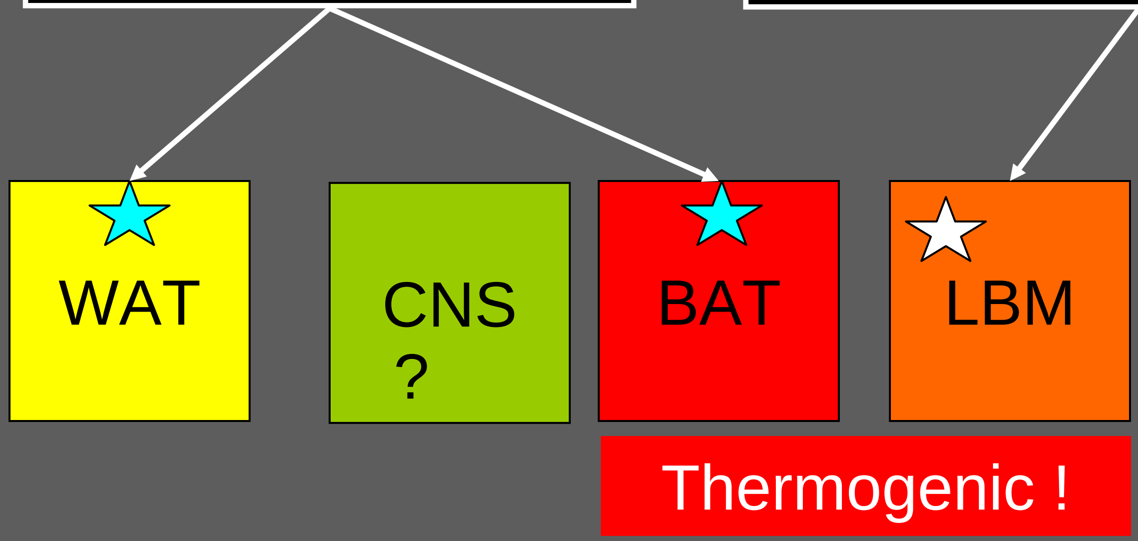
- *Does* remove variation in body mass (or lean mass / fat mass) from treatment group mean VO_2 values

- Requires no dubious prior assumptions — and even if the "weights" assigned to LBM and FM are questionable, the precision of mean VO_2 estimates is improved

A signature feature of the data set: high precision measurement of body fat and lean tissue by MRS

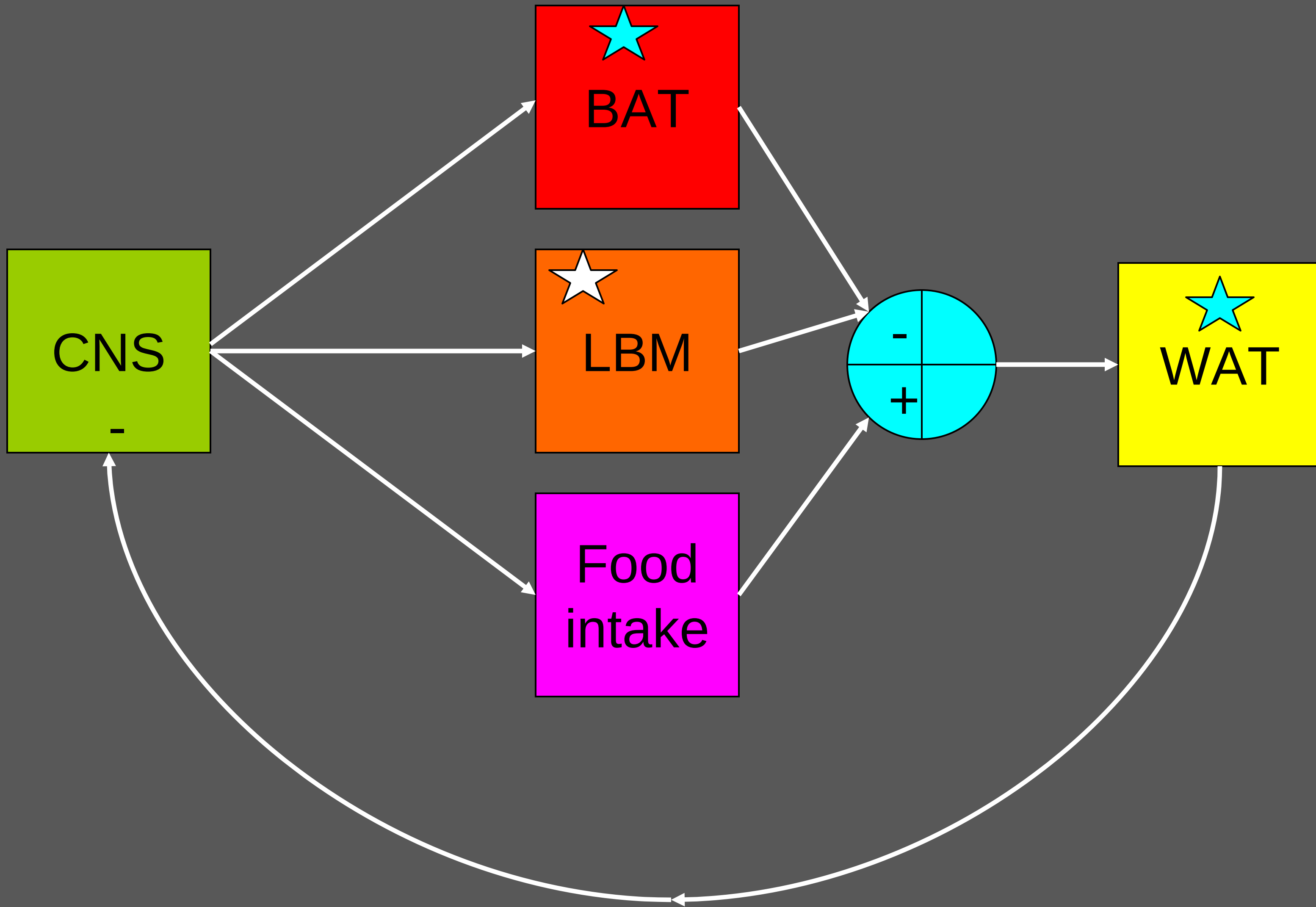
But MRS includes these in the fat measurement!

MRS provides a very good measure of muscle tissue



Implies that FM_{MRS} is a proxy for BAT mass/activity

Coupling of BAT and LBM themogenesis to adiposity by negative feedback regulation implies | LBM_{MRI}| *and* |FM_{MRI}| are determinants of VO_2



Theoretical relationship that linking per-kg VO_2 values for fat mass (B_{FM}), and lean body mass (B_{LBM}) to the per-kg value for body mass (B_{BM})

$$\text{Given that fat mass} = \left(\frac{\%\ \text{Fat}}{100}\right)(\text{Body mass})$$

$$\text{Lean body mass} \approx \left(1 - \frac{\%\ \text{Fat}}{100}\right)(\text{Body mass})$$

It is easy to show that

$$\text{Theoretical } B_{BM} = \left[(B_{FM} - B_{LBM})\left(\frac{\%\text{Fat}}{100}\right) + B_{LBM}\right](\text{Body mass})$$

Point: *B* estimates for body mass are weighted averages of the *B* values for fat mass and lean body mass

	Estimated contribution to VO2 (ml/kg tissue/min)	
Tissue	Minimum light cycle VO_2	24-h average VO_2
Lean body mass	21.7 ± 8.8	42.9 ± 10.7
Fat mass	30.7 ± 5.4	34.8 ± 6.4
Body mass	27.8 + 4.3	36.9 + 4.8
Theoretical Body Mass assuming 23% fat	23.8	41.0