Skeletal Muscle Insulin Resistance in Obesity and Diabetes. The Contributions of Jerry Reaven and the Role of Muscle Bioenergetics and Physical Inactivity

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No Disclosures

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OBITUARIES

Gerald “Jerry” M Reaven: the “father of insulin resistance”

Bob Roehr

Washington, DC

JCI The Journal of Clinical Investigation

Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes

Shiao-Wei Shen, … , Gerald M. Reaven, John W. Farquhar

J Clin Invest. 1970;49(12):2151-2160. https://doi.org/10.1172/JCI106433.

Research Article

A technique was devised for a more accurate measurement than has been heretofore possible of one of the factors responsible for hyperglycemia in the complex syndrome of diabetes. This factor is termed impedance and represents the tissues' insensitivity or resistance to insulin-mediated glucose uptake. It was measured by use of steady-state exogenous insulin and glucose infusions during a period of pharmacological suppression of endogenous insulin secretion. Endogenous new glucose production was also inhibited. Impedance as calculated is a direct function of steady-state glucose concentrations, since exogenous insulin concentrations were similar in all studies. Two groups of normal weight subjects were studied. One had maturity onset latent diabetes, and the other (matched for age, weight, and per cent adiposity) was normal. Impedance was closely reproducible in the same individual and remained relatively constant during prolonged infusions. The diabetics had average infusion glucose concentrations (and thus impedance) 68% higher than the normal group, and it is of note that their previously measured glucose intolerance differed by a similar degree; that is, the diabetic's intolerance (as defined by mean weighted plasma glucose response after oral glucose) was 52% greater than that of the normal individuals.

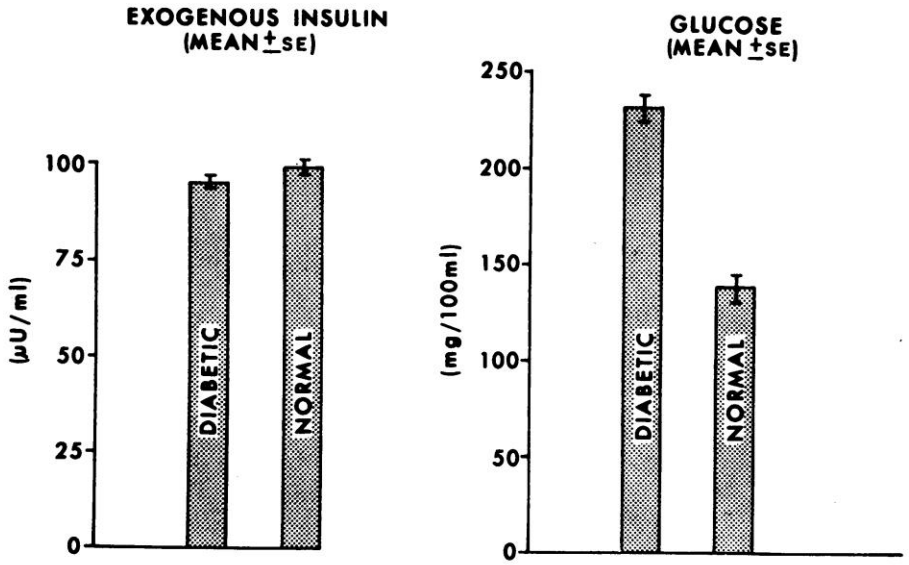


FIGURE 2 Steady-state plasma glucose and insulin concentrations of standard infusion studies in normal and diabetic subjects.

TABLE II

Results of Standard Infusion Studies

Subjects	Glucose infusion rate (V)	Plasma glucose*	Plasma insulin*	Impedance‖
	mg/kg per min	*mg/100 ml*	*μU/ml*	
Diabetics				
T. S.	5.9	241 ±4	91 ±4	40.8
T. W.	6.4	236 ±6	107§	36.9
C. H.	6.2	211 ±3	86 ±5	34.0
L. K.	6.2	236 ±5	94 ±11	38.1
F. R.	6.0	201 ±6	90 ±3	33.5
E. H.	6.0	231 ±6	86 ±5	38.5
G. R.	6.0	268 ±11	95§	44.7
Mean	6.1	232	93	38.1
Range	5.9–6.4	201–268	86–107	33.5–44.7
Normals				
V. B.	5.9	154 ±4	96 ±5	26.1
R. K.	6.0	118 ±7	112§	19.7
H. K.	5.9	158 ±4	93§	26.8
W. A.	5.9	140 ±6	94 ±4	23.7
H. M.	6.2	145 ±4	90 ±6	23.4
D. D.	6.1	149 ±3	87§	24.4
G. W.	6.0	99 ±4	110 ±6	16.5
Mean	6.0	138	97	22.9
Range	5.9–6.1	99–158	87–112	16.5–26.8

$V = k_u \times G$

Where:

V = rate of glucose uptake (set by infusion)

k_u = uptake constant, $1/k_u$ = "impedance"

G = plasma glucose concentration

WE WILL RETURN TO THIS MODEL

Banting Lecture 1988

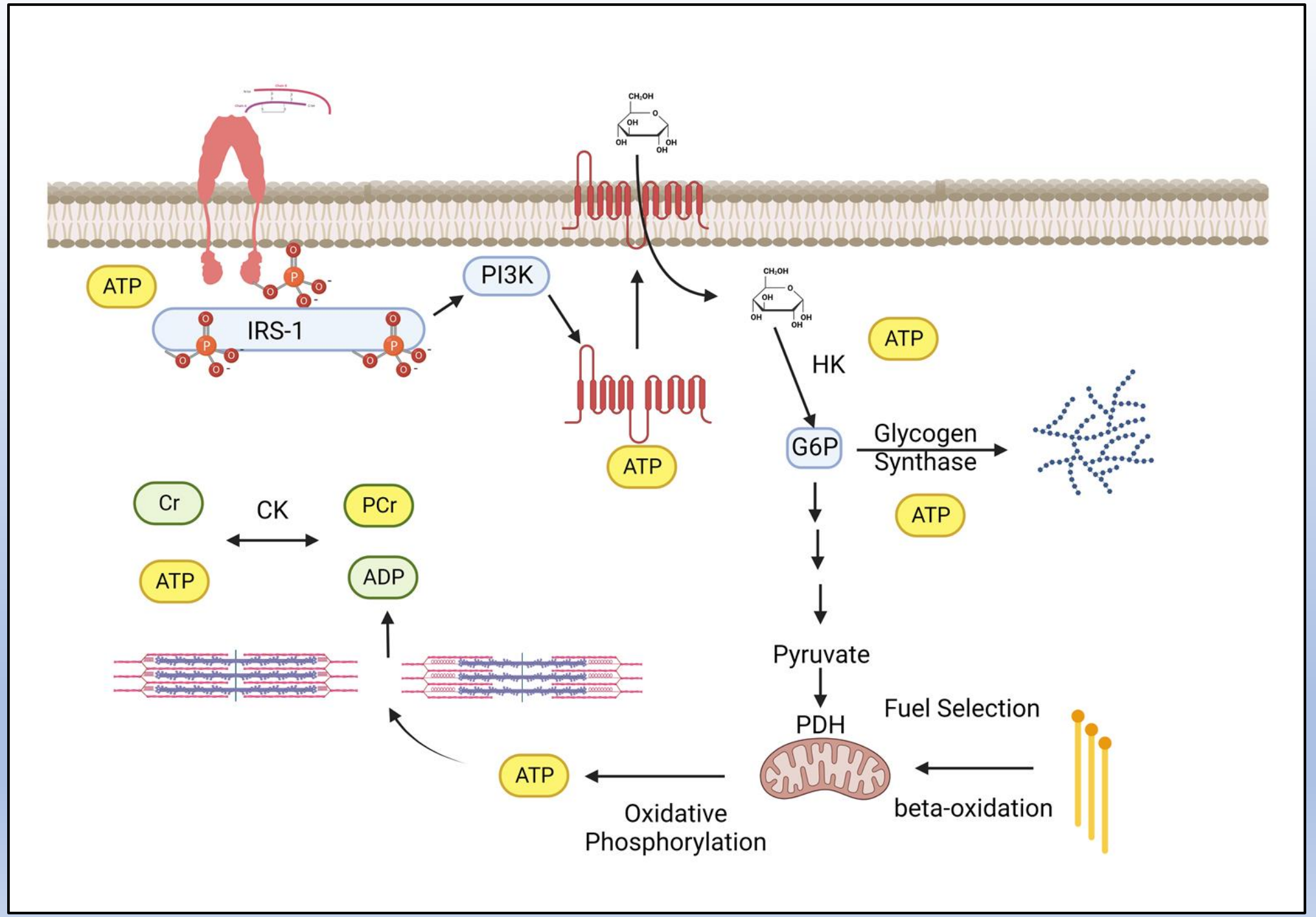
Role of Insulin Resistance in Human Disease

GERALD M. REAVEN

TABLE 1
Syndrome X

Resistance to insulin-stimulated glucose uptake
Glucose intolerance
Hyperinsulinemia
Increased very-low-density lipoprotein triglyceride
Decreased high-density lipoprotein cholesterol
Hypertension

Dr. Reaven's ideas led to many years of research into the molecular mechanisms of insulin resistance in skeletal muscle

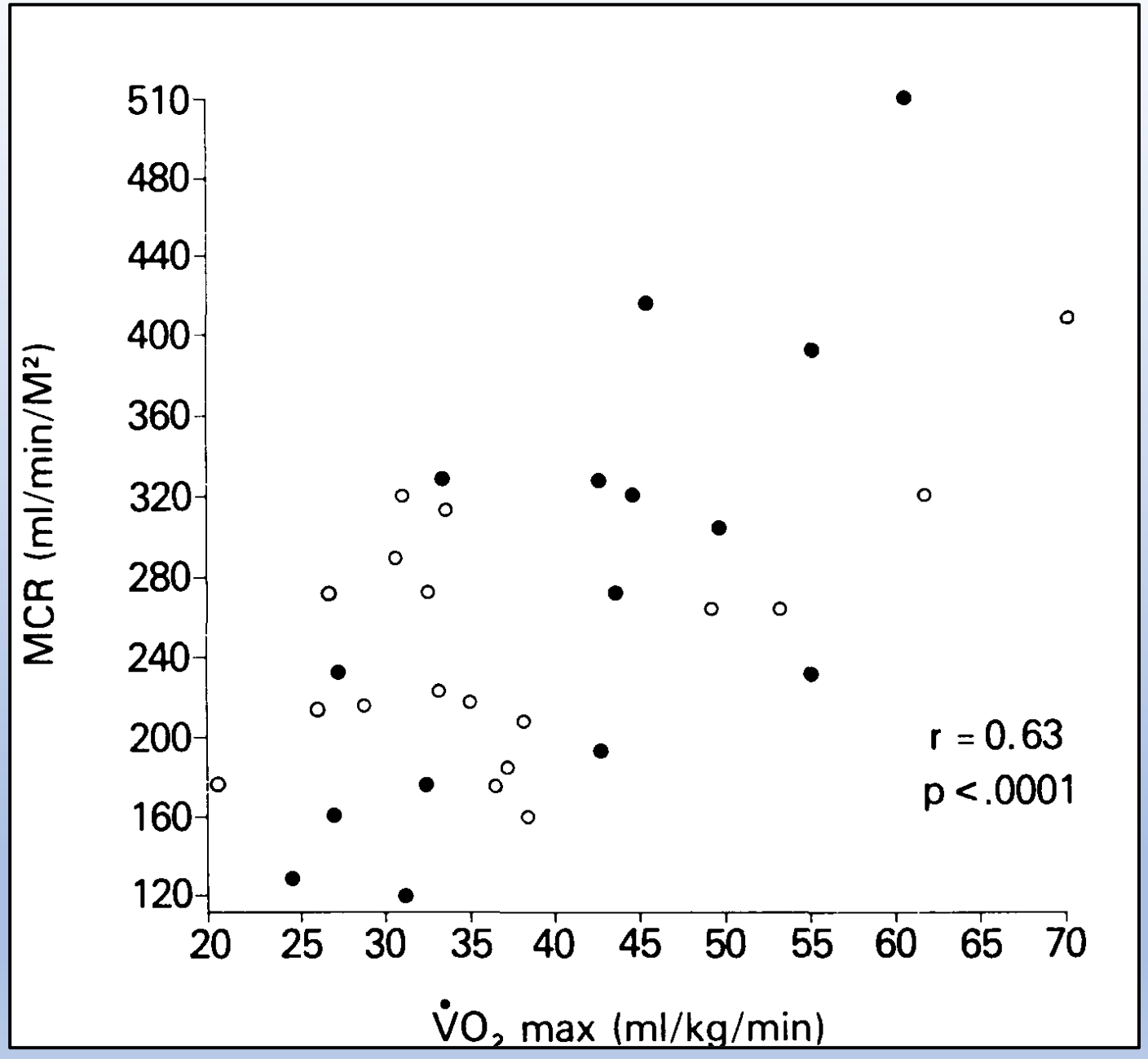


“Abnormalities” have been identified in nearly all aspects of insulin action in skeletal muscle, forcing consideration of common mechanisms. In this context, many researchers have focused on the role of functional mitochondrial content in insulin sensitivity.

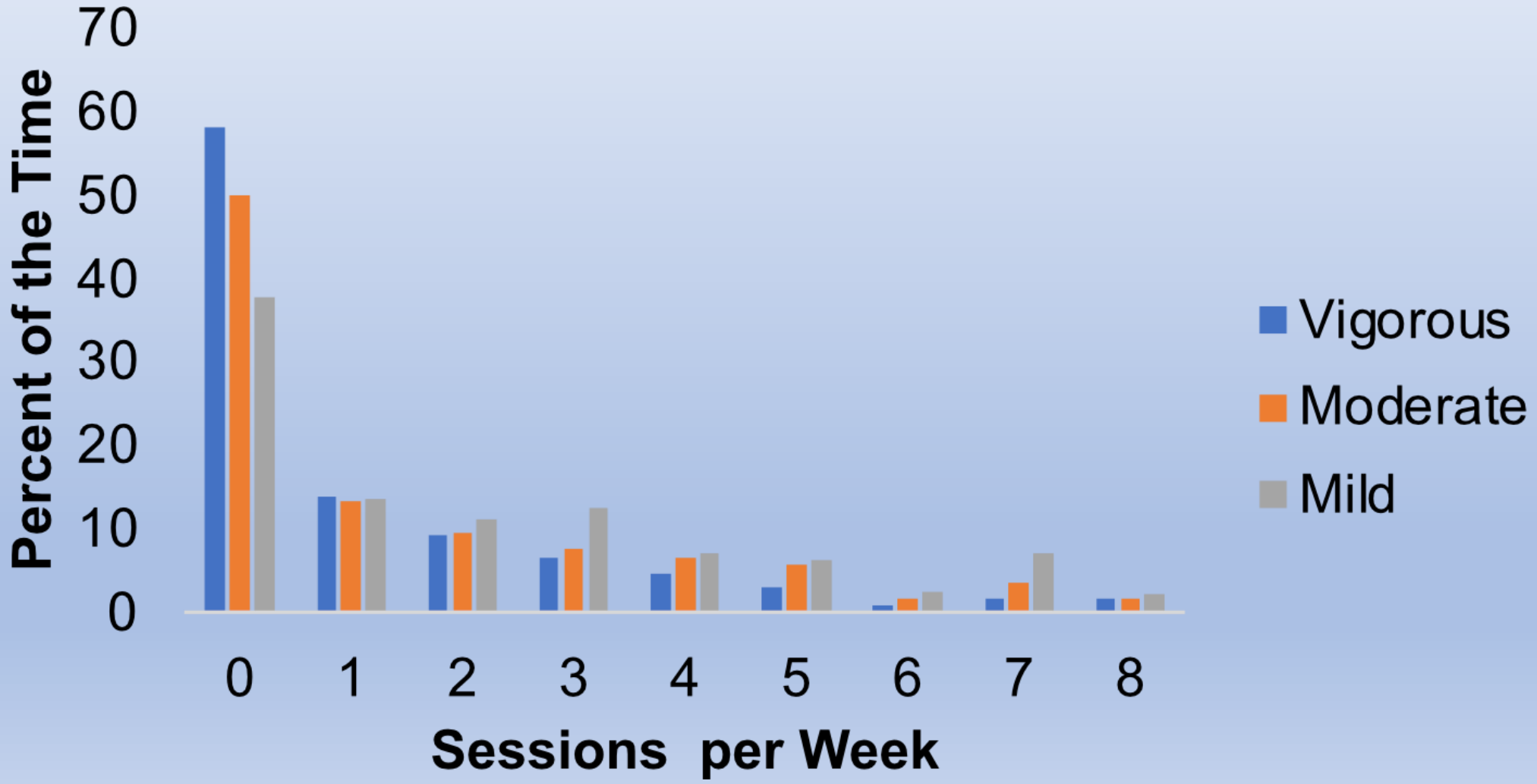
Demonstration of a Relationship Between Level of Physical Training and Insulin-stimulated Glucose Utilization in Normal Humans

MARK ROSENTHAL, W. L. HASKELL, ROBERT SOLOMON, ANDERS WIDSTROM, AND GERALD M. REAVEN

Aerobic capacity and insulin sensitivity are intimately related.



What proportion of adults engage in physical activity lasting at least 15 minutes? Data from over 1000 Latino patients in *El Banco por Salud*.



Is there evidence that muscle bioenergetics and thermodynamics are a primary regulator of insulin sensitivity? What would be the mechanism, and what did Jerry Reaven contribute to this idea?

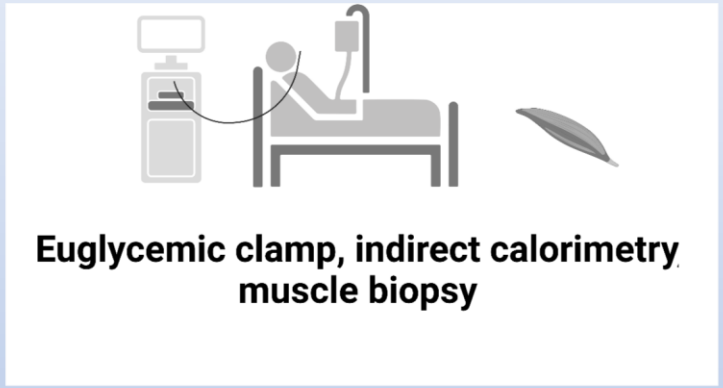
Keep this equation from Reaven in mind:

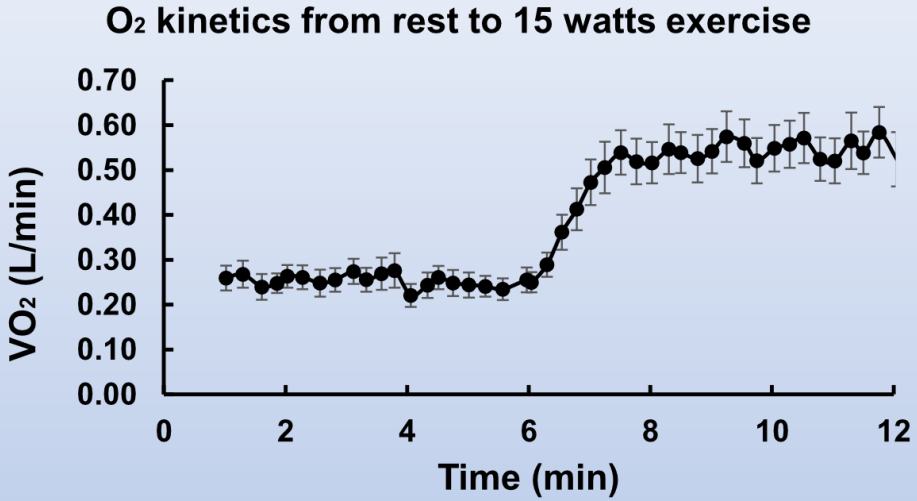
$V = k_u \times G$

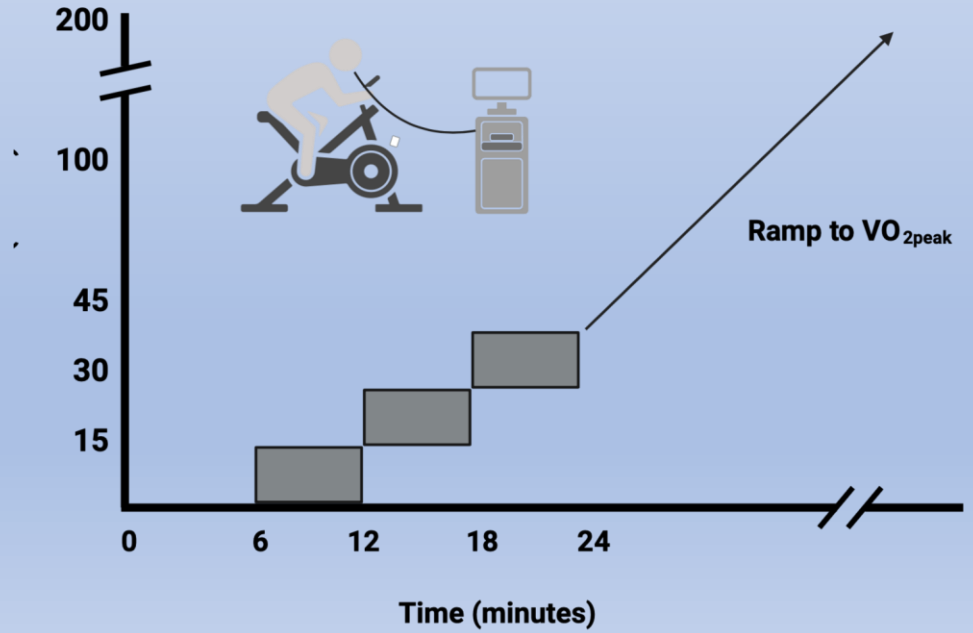
What about mitochondrial content in skeletal muscle in insulin resistance?

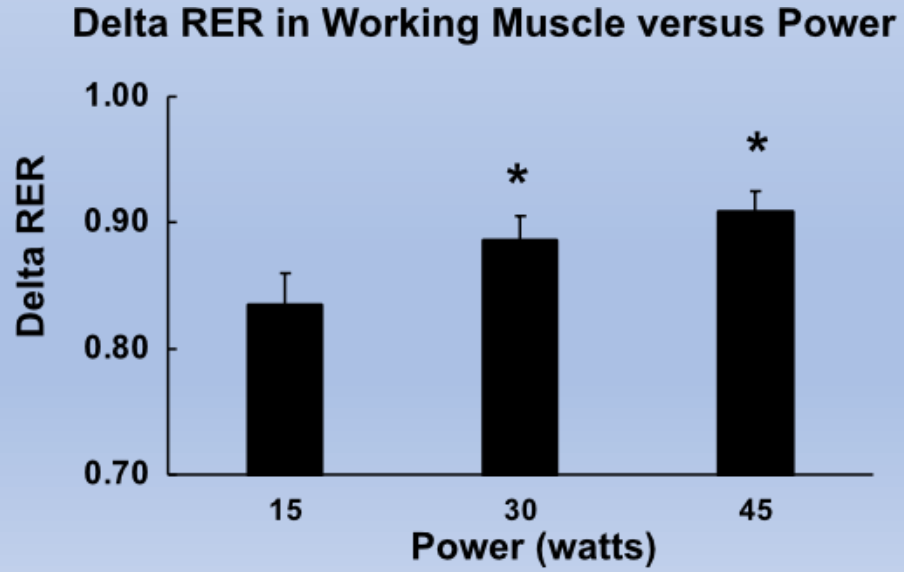
- Does mitochondrial abundance alter fuel selection and metabolic flexibility, an important aspect of insulin resistance?
- Does mitochondrial abundance alter the thermodynamic state of muscle?

Can mitochondrial content influence fuel selection? A study in sedentary people.



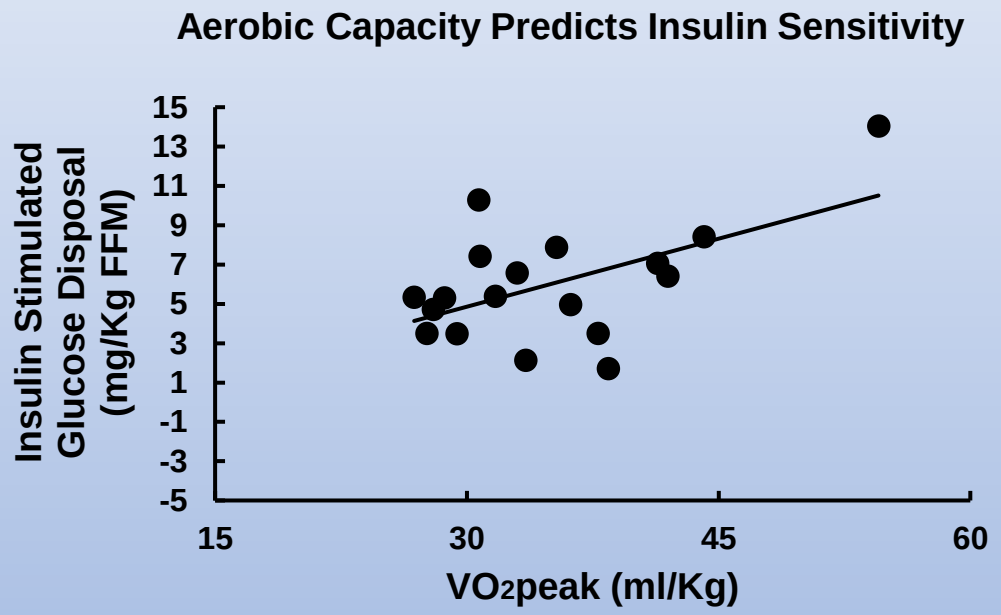


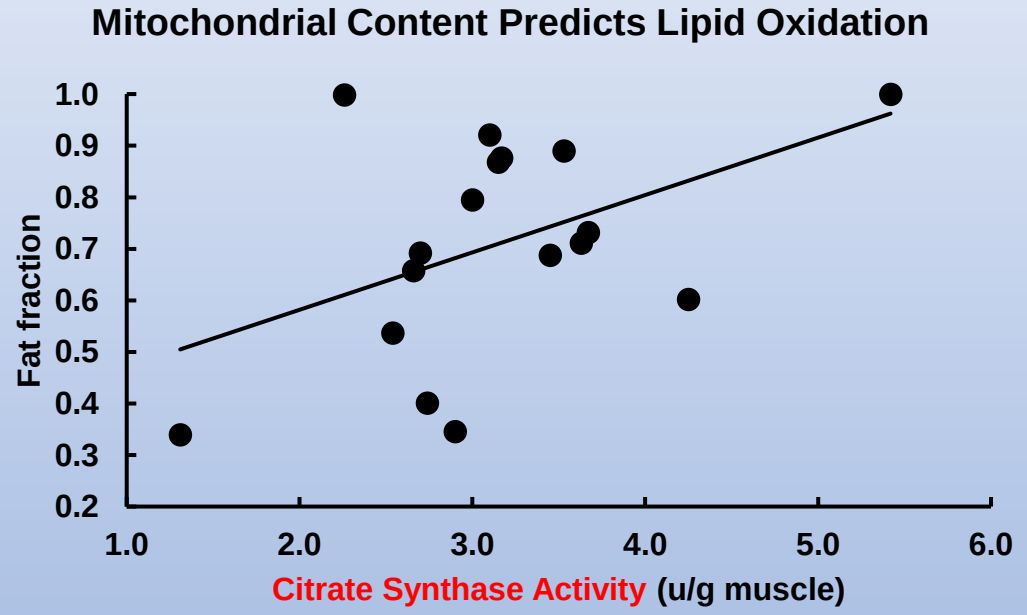




Barakati et al., in press, Metabolic Syndrome and Related Disorders

Mitochondrial content predicts fuel selection in mildly exercising muscle



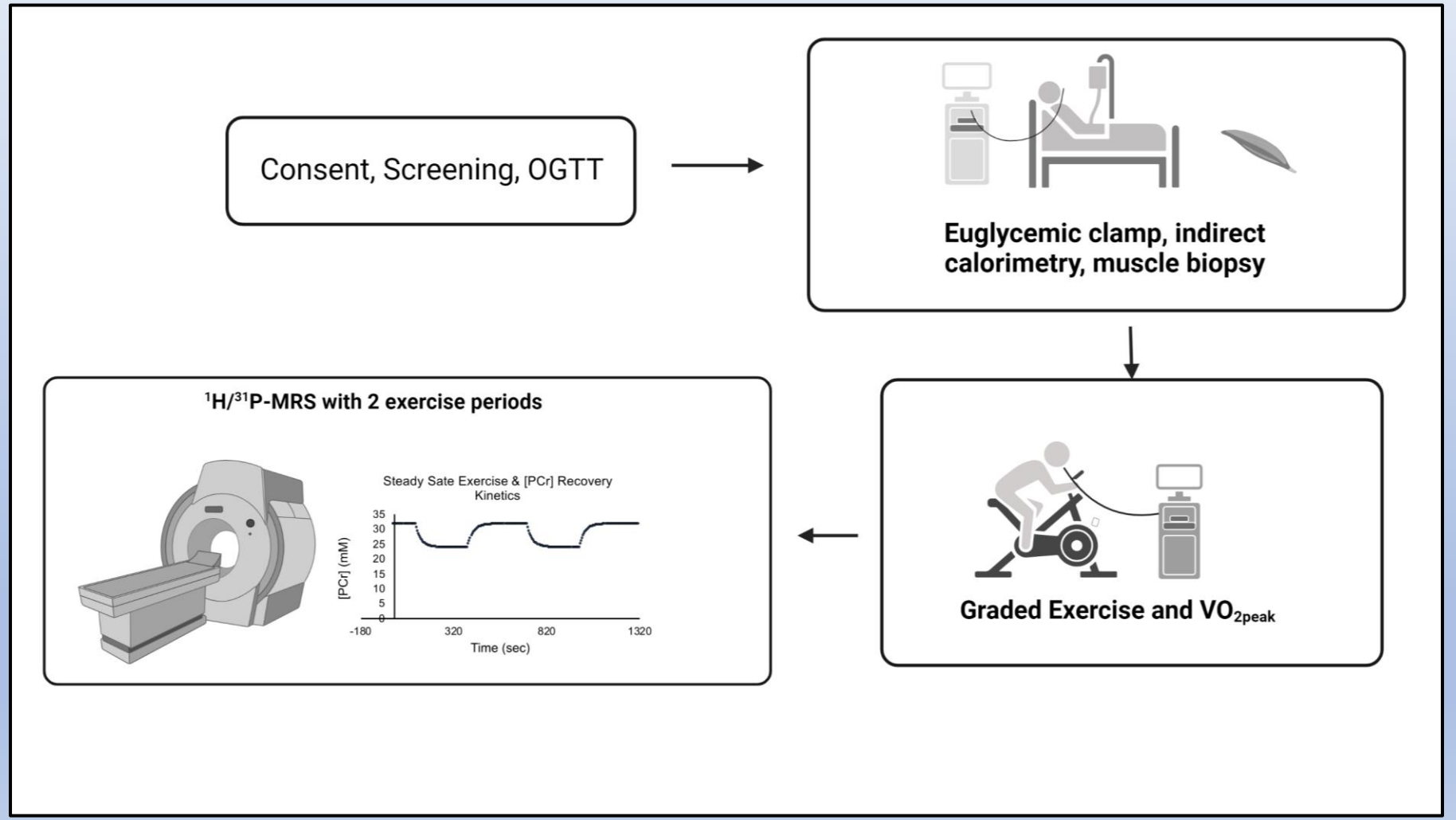


Barakati et al., in press, Metabolic Syndrome and Related Disorders

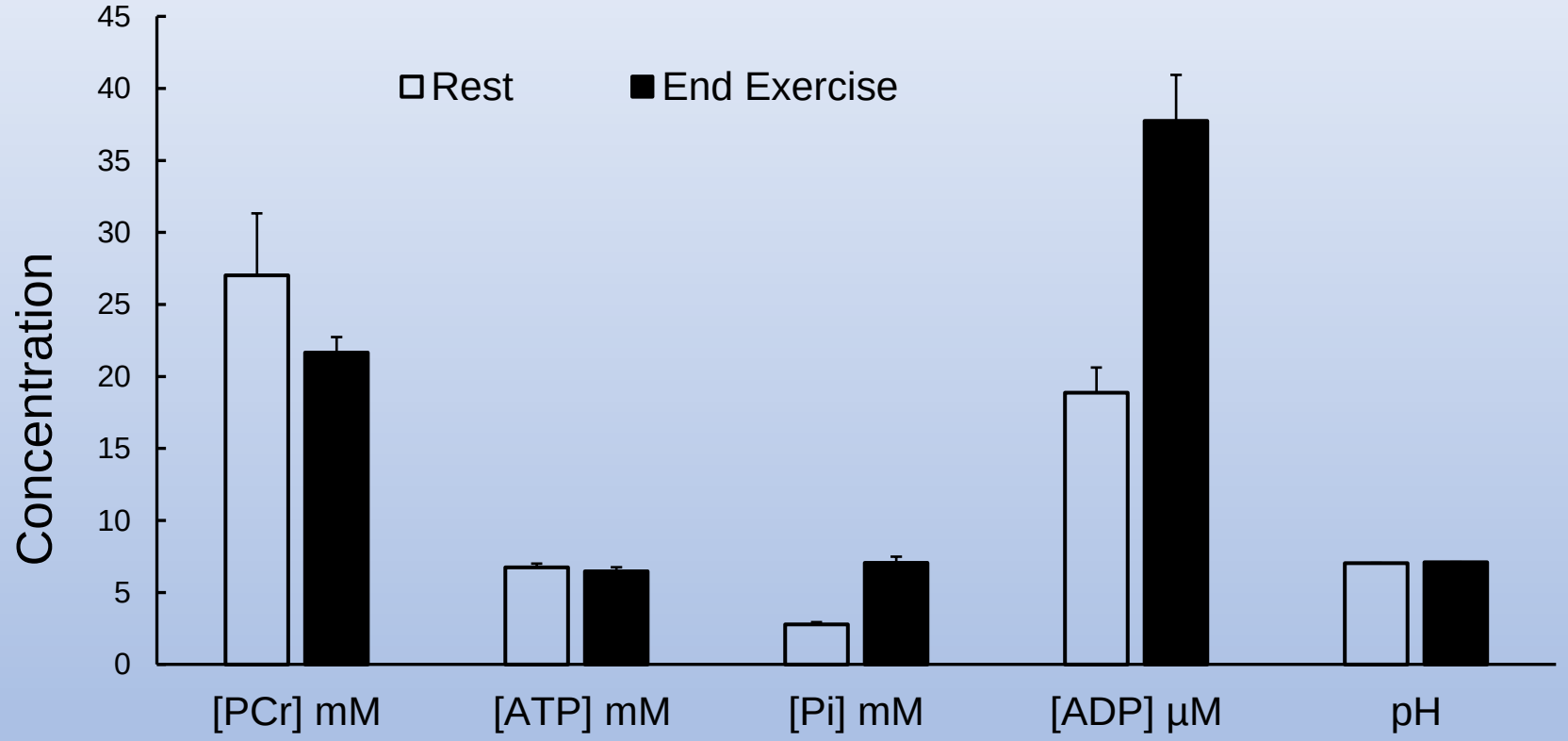
Thermodynamics: What is meant by "thermodynamics" in muscle?

- In this case, we refer to the free energy of hydrolysis of ATP, or the Gibbs free energy, ΔG_{ATP}.
- Although many people think of ATP as having a single high energy value for driving reactions, that is not the case in skeletal muscle or other tissues that have creatine kinase, a reaction linking ATP, ADP, and Pi via creatine and phosphocreatine. The force with which ATP can drive a reaction varies physiologically.
- $\Delta G_{ATP} = RT \times \ln([ATP][Pi]/[ADP])$,
  - R = gas constant
  - T = temp in Kelvin
  - Units are kcal/mole or kJoules/mole

How can we estimate mitochondrial content and thermodynamics (ΔG_{ATP}) *in vivo?* ^{31}P-MRS studies



Effect of exercise on energy phosphates – these concentrations are used to calculate ΔG_{ATP}



Calibrated MRS must be used to determine energy phosphate concentrations individually.

Estimation of mitochondrial content by ^{31}P-MRS

Linear dependence of muscle phosphocreatine kinetics on total creatine content

RONALD A. MEYER
Departments of Physiology and Radiology, Michigan State University, East Lansing, Michigan 48824

MEYER, RONALD A. *Linear dependence of muscle phosphocreatine kinetics on total creatine content.* Am. J. Physiol. 257 (Cell Physiol. 26): C1149–C1157, 1989.—Phosphorus nuclear magnetic resonance (NMR) spectra and twitch tension were recorded during stimulation of gastrocnemius muscles of pentobarbital sodium-anesthetized rats which had been fed the creatine analogue β-guanidinopropionic acid (β-GPA, 2% diet) for periods from 0 (control) to 8 wk. Total creatine content of unstimulated muscles decreased by 42, 67, 82, and 88% compared with controls after 2-, 4-, 6-, and 8-wk feeding, respectively. The staircase effect observed in control muscles during 8 min of twitch stimulation at 0.25, 0.5, and 0.75 Hz was reduced after 2- to 8-wk β-GPA feeding. However, after 6- to 8-wk feeding, the twitch force at the end of 8 min of stimulation was not different from controls. The time constant for phosphocreatine (PCr) changes at the onset of and during recovery after stimulation was proportional to total creatine content. The relationship between PCr content and twitch rate times force at the end of stimulation was linear, with slope proportional to total creatine content. PCr content in β-GPA-fed animals was transiently greater during recovery than before stimulation, suggesting a regulatory effect of the inorganic phosphate released by hydrolysis of phosphorylated β-GPA. The results are consistent with linear models of respiratory control in which the creatine kinase reaction acts as a simple buffer of adenine nucleotide levels.

creatine, β-guanidinopropionic acid (β-GPA; Ref. 17). The analogue accumulates and is reversibly phosphorylated by creatine kinase in the muscle, but at a much slower rate than creatine (3). Thus the phosphorylated analogue cannot effectively serve any of the metabolic roles postulated for phosphocreatine, for example, in the creatine shuttle (1, 20), as an adenylate buffer or chemical capacitor (11, 13), or as a readily available source of inorganic phosphate (2). Several studies have compared the metabolism and performance of muscles of chronically β-GPA-fed vs. control rats (10, 12, 15, 19). These studies have generally concluded that PCr plays an important role during transitions from rest to exercise but not during steady-state exercise which can normally be maintained by aerobic metabolism (12, 15). Thus these studies suggested that the creatine shuttle is not a primary function of the creatine kinase system in skeletal muscle. However, previous studies only examined rats that were fed the analogue for several weeks so that maximal creatine depletion had occurred. During the long feeding period adaptations occur in fast-twitch muscle fibers (e.g., reduced fiber diameter, increased citrate synthetase, decreased glycogen phosphorylase; Refs. 17, 18) which might overcome a defect in steady-state performance caused by creatine depletion. Indeed, it might

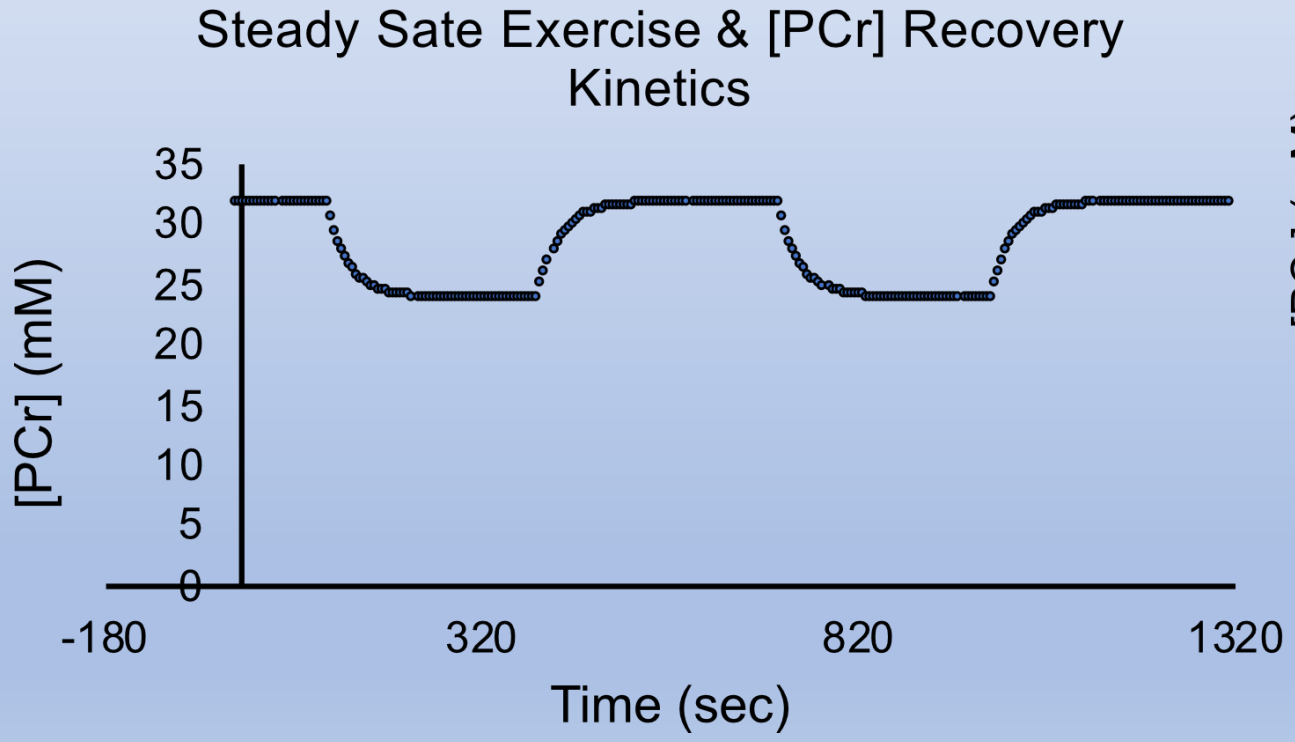
$\tau = R_{mito} \times$ [Total Creatine]

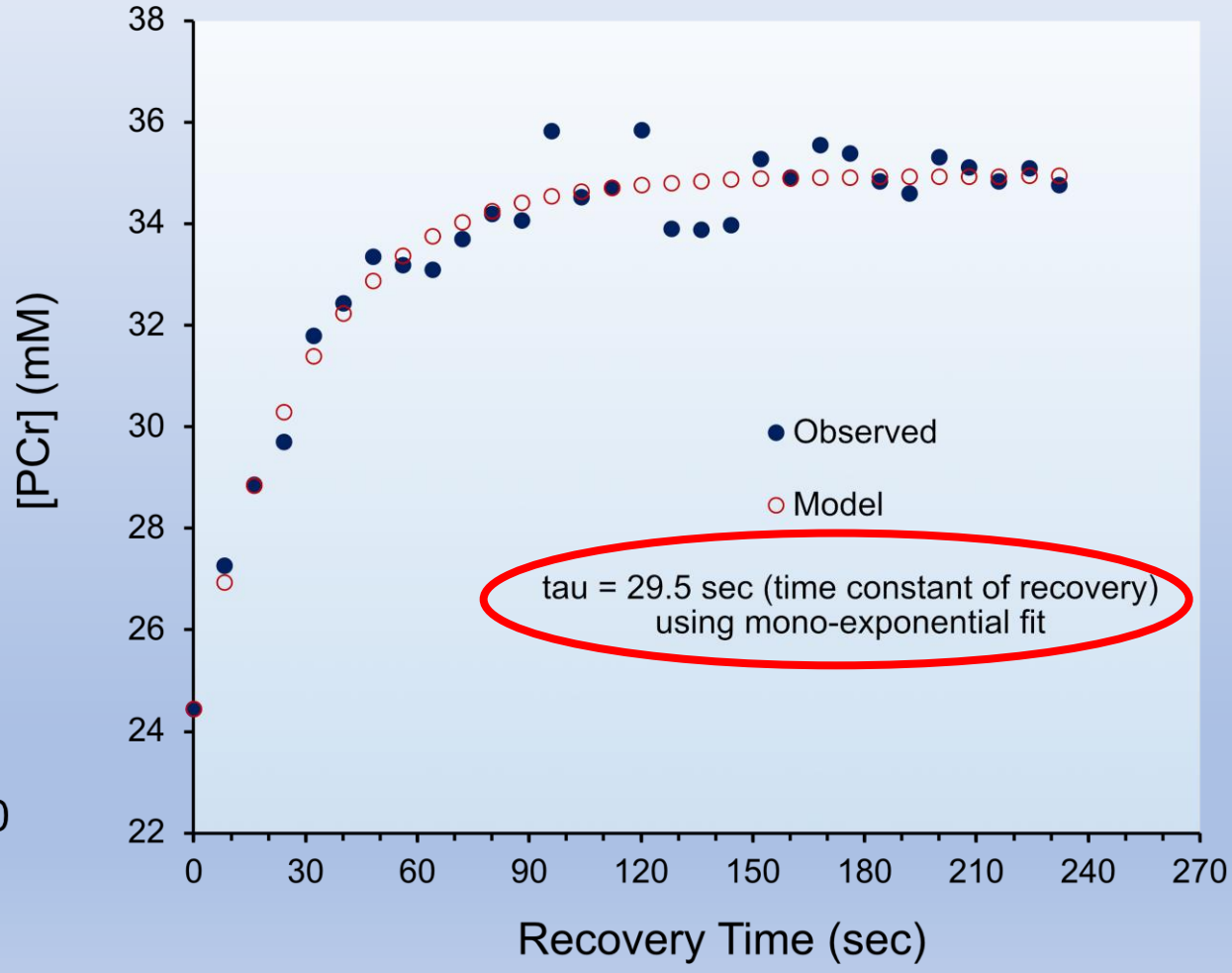
R_{mito} = resistance of mitochondria to energy flow

$1/R_{mito}$ = conductance to energy flow, which is proportional to mitochondrial functional content.

So τ is inversely proportional to mitochondrial content, modulated by the total creatine capacitor.

Recovery of phosphocreatine after exercise can be used to measure functional mitochondrial content *in vivo*.





Tau is a linear function of creatine and mitochondrial content

Am J Physiol Cell Physiol 294: C79–C87, 2008.
First published October 17, 2007; doi:10.1152/ajpcell.00138.2007.

Linear relation between time constant of oxygen uptake kinetics, total creatine, and mitochondrial content in vitro

Brian Glancy,[1] Thomas Barstow,[2] and Wayne T. Willis[1]

[1]*Department of Kinesiology, Arizona State University, Tempe, Arizona; and* [2]*Department of Kinesiology, Kansas State University, Manhattan, Kansas*

Submitted 3 April 2007; accepted in final form 11 October 2007

Glancy B, Barstow T, Willis WT. Linear relation between time constant of oxygen uptake kinetics, total creatine, and mitochondrial content in vitro. *Am J Physiol Cell Physiol* 294: C79–C87, 2008. First published October 17, 2007; doi:10.1152/ajpcell.00138.2007.—Following the onset of moderate aerobic exercise, the rate of oxygen consumption (J_o) rises monoexponentially toward the new steady state with a time constant (τ) in the vicinity of 30 s. The mechanisms underlying this delay have been studied over several decades. Meyer's electrical analog model proposed the concept that the τ is given by $\tau = R_m \cdot C$, where R_m is mitochondrial resistance to energy transfer, and C is metabolic capacitance, determined primarily by the cellular total creatine pool (TCr = phosphocreatine + creatine). The purpose of this study was to evaluate in vitro the J_o kinetics of isolated rat skeletal muscle mitochondria at various levels of TCr and mitochondrial protein. Mitochondria were incubated in a medium containing 5.0 mM ATP, TCr pools of 0–1.5 mM, excess creatine kinase, and an ATP-splitting system of glucose + hexokinase (HK). Pyruvate and malate (1 mM each) were present as oxidative substrates. J_o was measured across time after HK was added to elicit one of two levels of J_o (40 and 60% of *state 3*). At TCr levels (in mM) of 0.1, 0.2, 0.3, 0.75, and 1.5, the corresponding τ values (s, means ± SE) were 22.2 ± 3.0, 36.3 ± 2.2, 65.7 ± 4.3, 168.1 ± 22.2, and 287.3 ± 25.9. Thus τ increased linearly with TCr ($R^2 = 0.916$). Furthermore, the experimentally observed τ varied linearly and inversely with the mitochondrial protein added. These in vitro results consistently conform to the predictions of Meyer's electrical analog model.

mitochondrial resistance; hexokinase; creatine kinase

$$\tau = R_m \cdot C \qquad (1)$$

Theoretical analysis by Kushmerick (36) supports a critical assumption in the Meyer model that the creatine kinase (CK) reaction is maintained near equilibrium during aerobic ATP turnover. With this requirement satisfied, the high equilibrium constant along with the nearly linear relation observed between steady-state cellular free energy of ATP hydrolysis (ΔG_{ATP}) and mitochondrial J_o (9, 11, 34, 43), together dictate that a predictable PCr net breakdown (a discharge of capacitance) must occur in the transition from one (lower) steady-state ATP turnover to another (higher) rate. That negligible C exists within the mitochondrion itself, e.g., in the matrix, electron transport chain, or proton gradient, has been shown by Wojtczak et al. (66, 67). Thus the strictest interpretation of the Meyer model predicts that any impact mitochondria make on the kinetics of O_2 uptake would be evident in the steady-state J_o-ΔG_{ATP} relation. In contrast, TCr would be predicted to affect neither the energetic forces nor flows once steady state is achieved.

Meyer experimentally supported his model by showing that progressive depletion of the Cr pool of rat skeletal muscle with dietary β-guanidinopropionic acid (β-GPA) yields faster PCr kinetics, as assessed with ^{31}P-nuclear magnetic resonance spectroscopy, at the onset of elevated contractile demand (44). More recently, the model has been supported in computer models of oxidative phosphorylation (35). In addition, Kindig

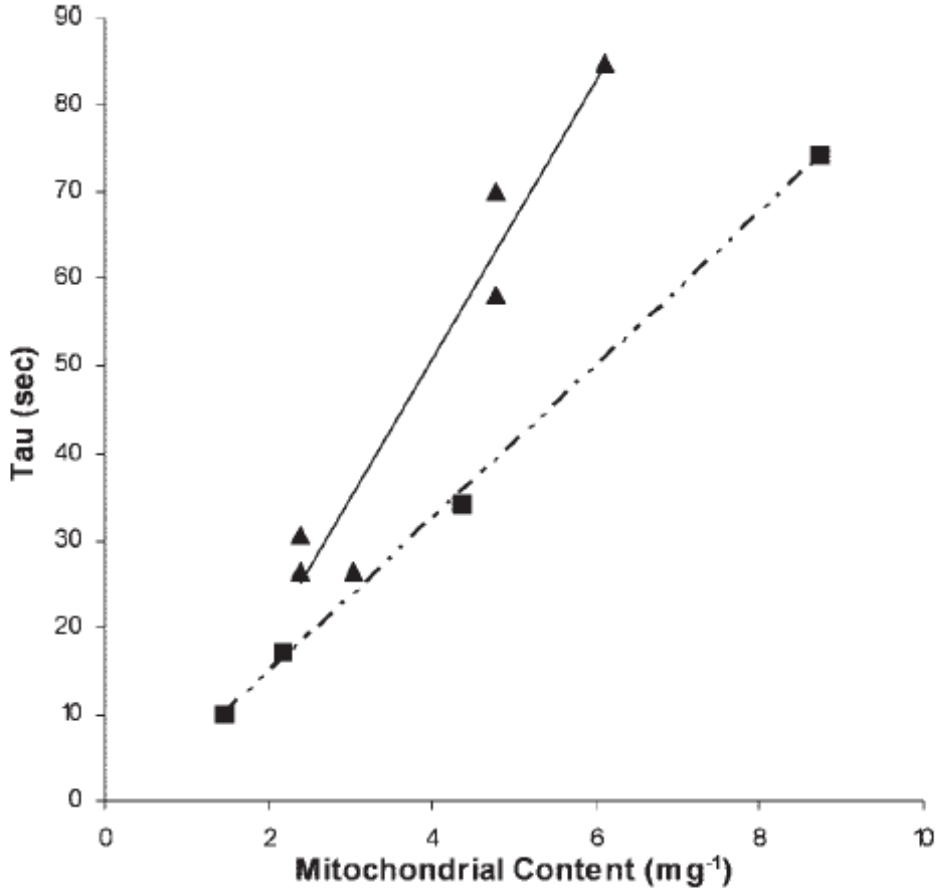
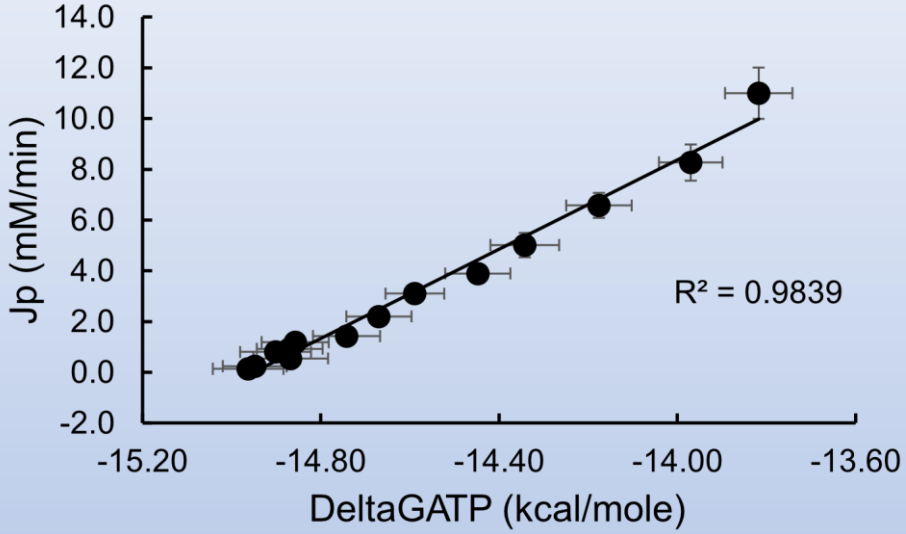
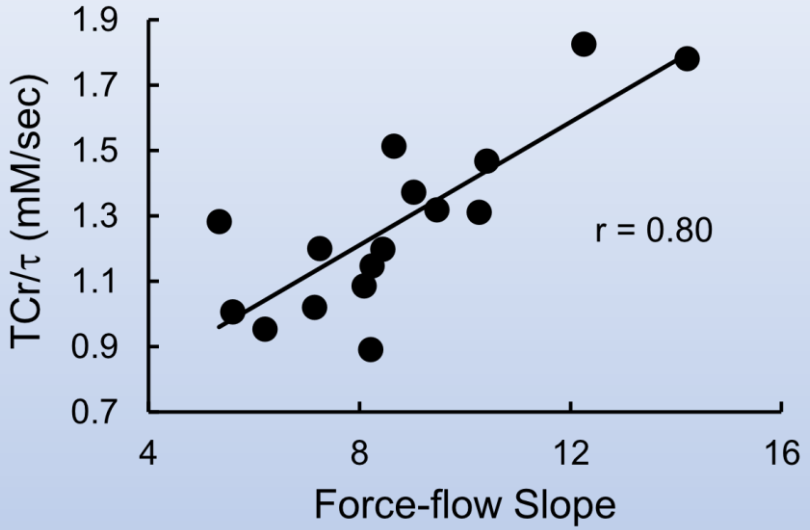
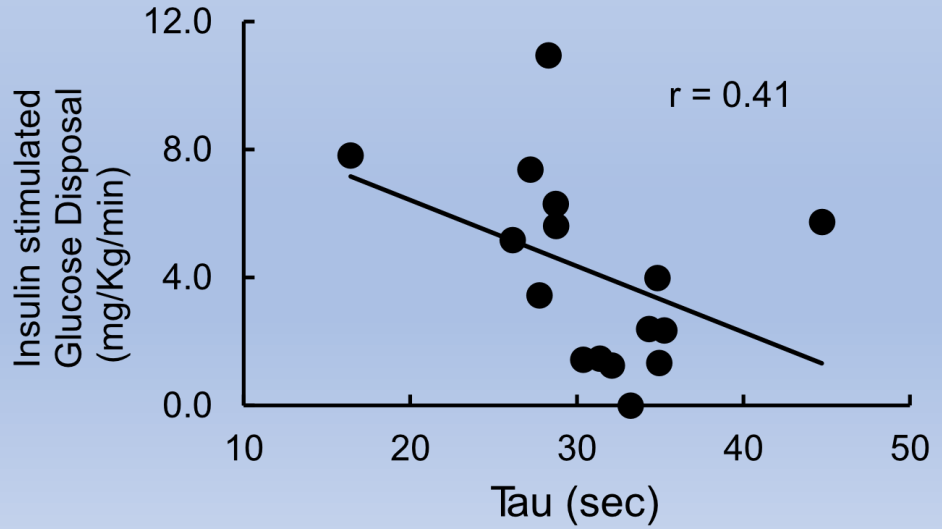


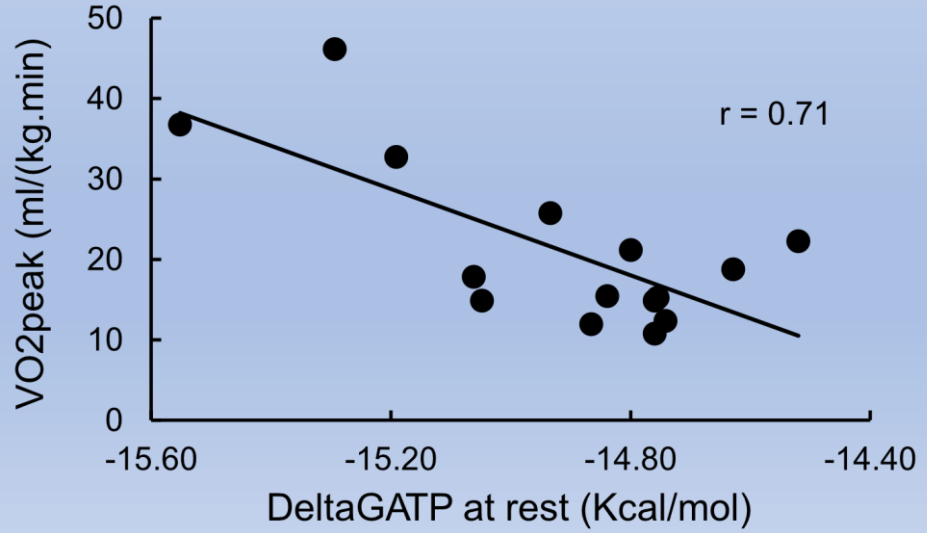
Fig. 3. Measured τ at varying levels of mitochondrial content. Triangles represent 0.3 mM TCr ($n = 6$), $R^2 = 0.941$. Squares represent 0.15 mM TCr ($n = 4$), $R^2 = 0.999$.

Validation of the Model

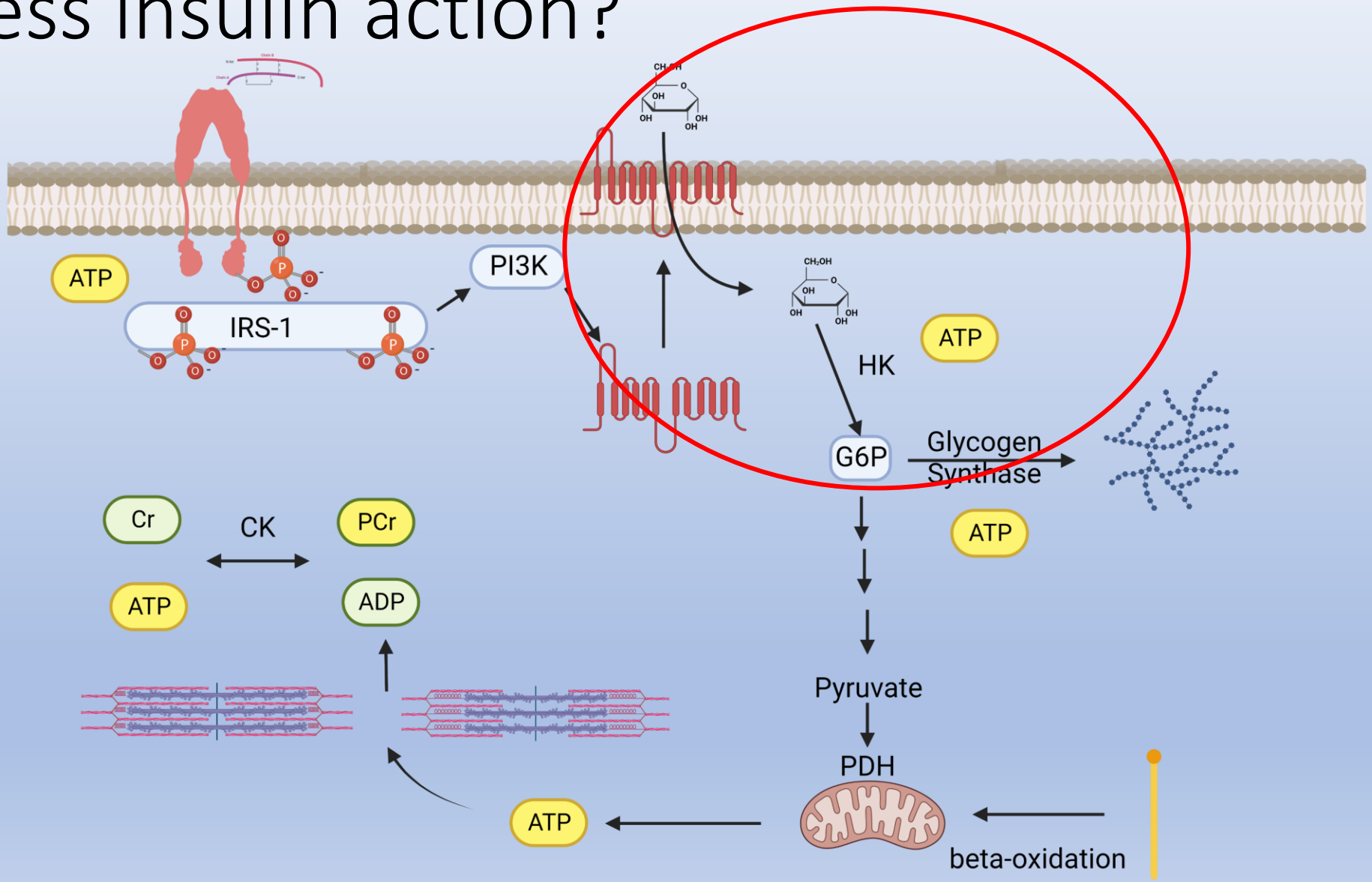








Can we use thermodynamic principles to assess insulin action?



Glucose uptake in muscle

- Two reactions:
- $Glu_{out} \rightleftharpoons Glu_{in}$
- $Glu_{in} + ATP \longrightarrow G6P + ADP$
- How do we calculate ΔG (the driving force) for these reactions?

Calculation of free energy (driving force) of reactions

- $\Delta G_{glut} = RT \times \ln([G_{in}]/[G_{out}])$
- $\Delta G_{HK} = RT \times \ln([ADP][G6P]/[ATP][G_{in}])$

- Knowing Jss (rate of reaction), ΔG, and []'s, we can calculate conductance (and hence resistance) using a version of Ohm's law.

Ohm's Law applied to glucose metabolism

- Ohm's Law –
  - Current (flux rate) = Voltage (pressure or change in free energy)/Resistance
  - Conductance (C) = 1/Resistance
  - Conductance = flux rate (current)/voltage (change in free energy)
- So, you can think of conductance as the ease with which a reaction takes place, calculated as:
  - Conductance = Jss/ΔG
- Using rates (Jss) and concentrations from DeFronzo's triple tracer studies, the conductance of the glucose transport and hexokinase steps of glucose uptake can be calculated.

Triple Tracer Model

Am J Physiol Endocrinol Metab 292: E92–E100, 2007.
First published August 8, 2006; doi:10.1152/ajpendo.00617.2005.

Muscle glucose transport and phosphorylation in type 2 diabetic, obese nondiabetic, and genetically predisposed individuals

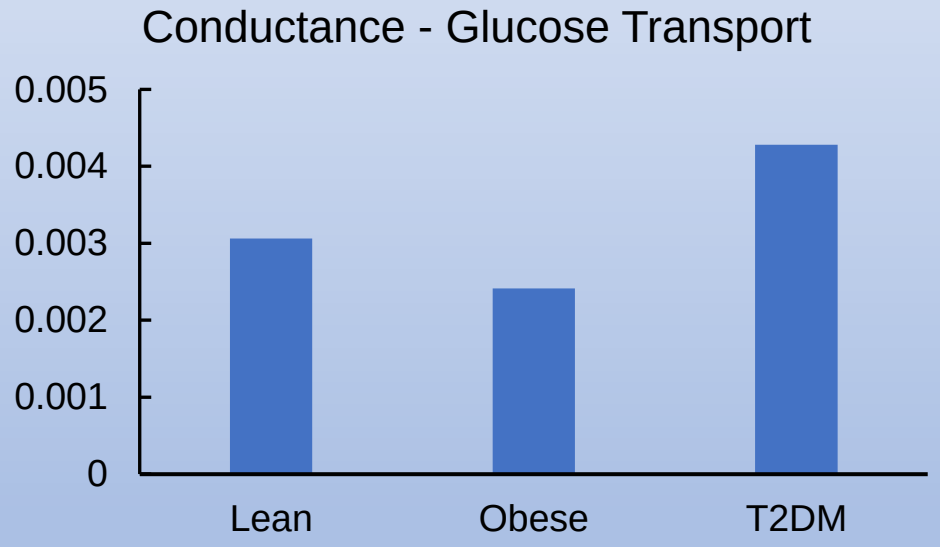
Merri Pendergrass,[1] Alessandra Bertoldo,[2] Riccardo Bonadonna,[3] Gianluca Nucci,[2] Lawrence Mandarino, Claudio Cobelli,[2] and Ralph A. DeFronzo[1]

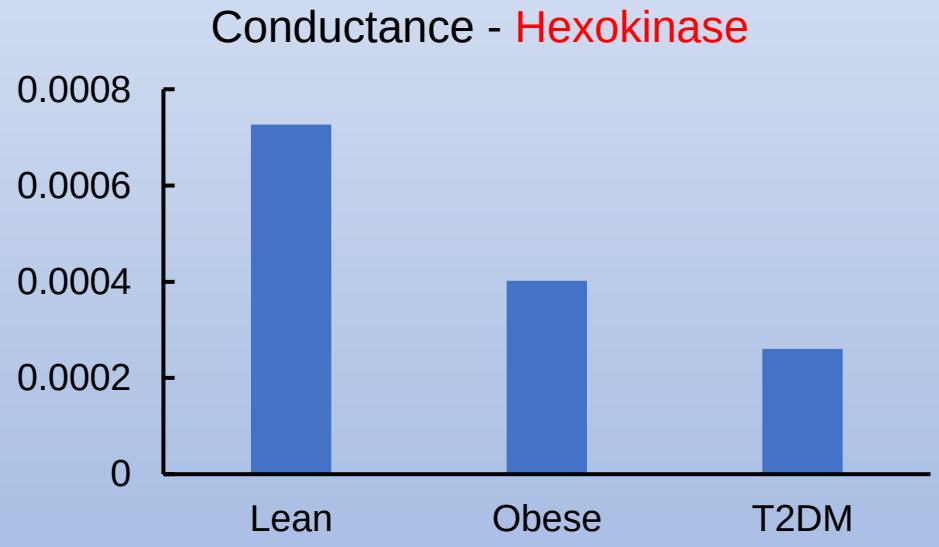
[1]University of Texas Health Science Center, San Antonio, Texas; [2]Department of Information Engineering, University of Padova, Padua; and [3]Division of Endocrinology and Metabolic Diseases, Verona School of Medicine, Verona, Italy

Submitted 6 December 2005; accepted in final form 21 July 2006

- Arterial injection of:
  - D-[12C]mannitol (not transportable by GLUTs)
  - 3-O-[14C]methyl-D-glucose (transportable but not metabolizable)
  - D-[3-3H]glucose (transportable and metabolizable by hexokinase)
- Following decay of these tracers using a compartmental model allows estimation of rates of glucose transport, glucose phosphorylation, interstitial [G_{out}] and intracellular [G_{in}]
- [G6P] estimated by MRS (Shulman) and others
- [ADP] and [ATP] from our data, assuming insulin does not affect []’s

Conductance at the glucose transport and hexokinase reactions





Remember Jerry Reaven's mathematical formulation for glucose uptake?

$V = k_u$ X G, where:

V = rate of glucose uptake (set by infusion)

$1/k_u$ = "impedance"

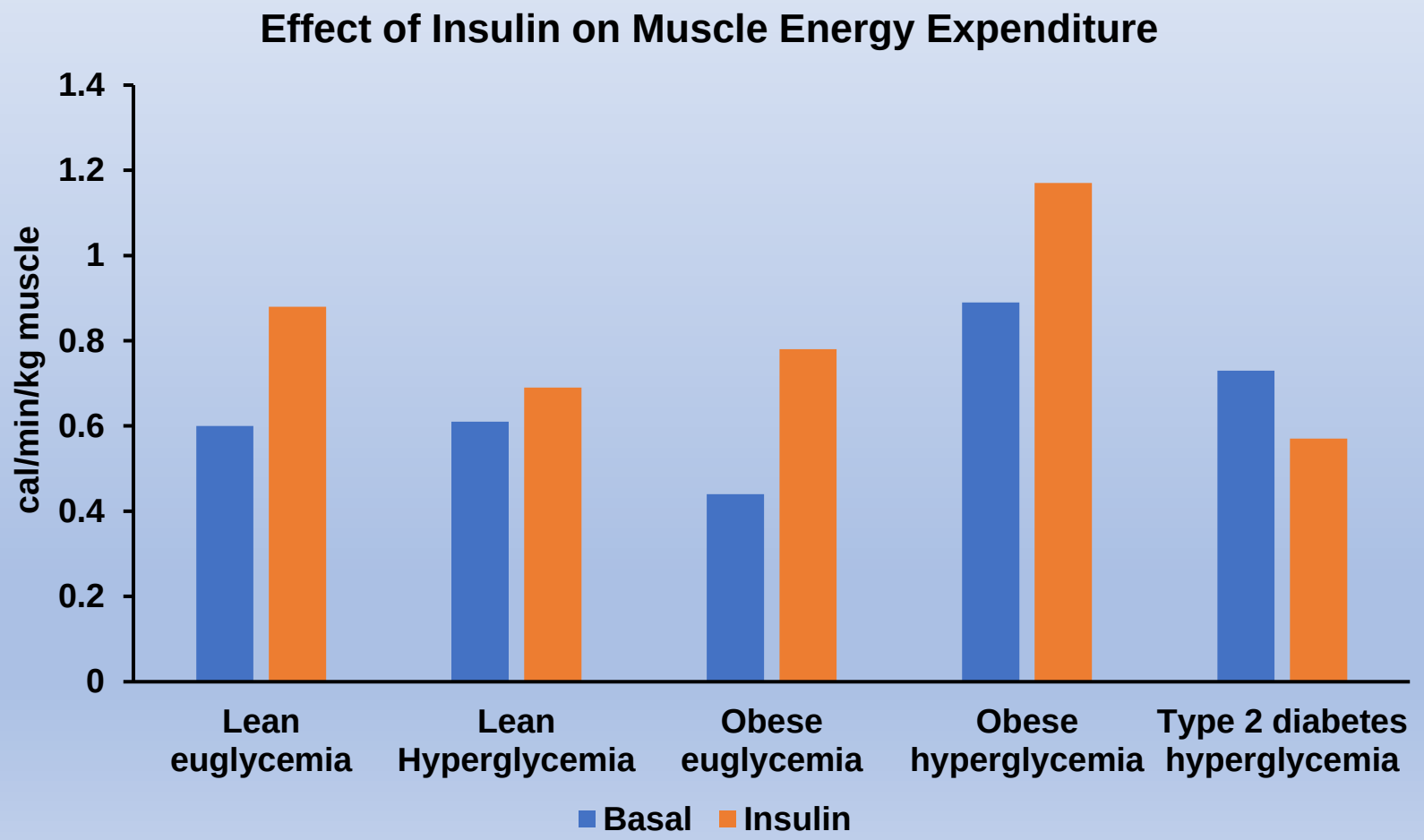
G = plasma glucose concentration

He had it almost right but did not take into account the principal that thermodynamics and kinetics are two sides of the same coin, so he did not consider the driving force for glucose transport or phosphorylation. In fairness he was ahead of his time.

Accounting for thermodynamics is critical for understanding metabolism in skeletal muscle since thermodynamic considerations govern reactions where ΔG_{ATP} provides the driving force.

Can altered thermodynamics due to lower mitochondrial content affect insulin action? *In other words, can we explain insulin resistance from first principles, independent of molecular mechanism?*

Insulin raises energy expenditure in skeletal muscle – results of leg balance studies



The increase in energy expenditure due to insulin is equivalent to mild exercise

	Basal		Insulin	
	Watts/kg muscle	Watts per whole body	Watts/kg muscle	Watts per whole body
Lean euglycemia	0.60	16.8	0.88	24.5
Lean Hyperglycemia	0.61	16.9	0.69	19.2
Obese euglycemia	0.44	12.3	0.78	21.8
Obese hyperglycemia	0.89	24.9	1.17	32.8
Type 2 diabetes hyperglycemia	0.73	20.3	0.57	15.9

The rate of a reaction is a nearly linear function of the thermodynamic driving force (ΔG_{ATP})

Biochimica et Biophysica Acta, 591 (1980) 488—493

BBA Report

BBA 41332

LINEAR RELATION BETWEEN RATE AND THERMODYNAMIC FORCE IN ENZYME-CATALYZED REACTIONS

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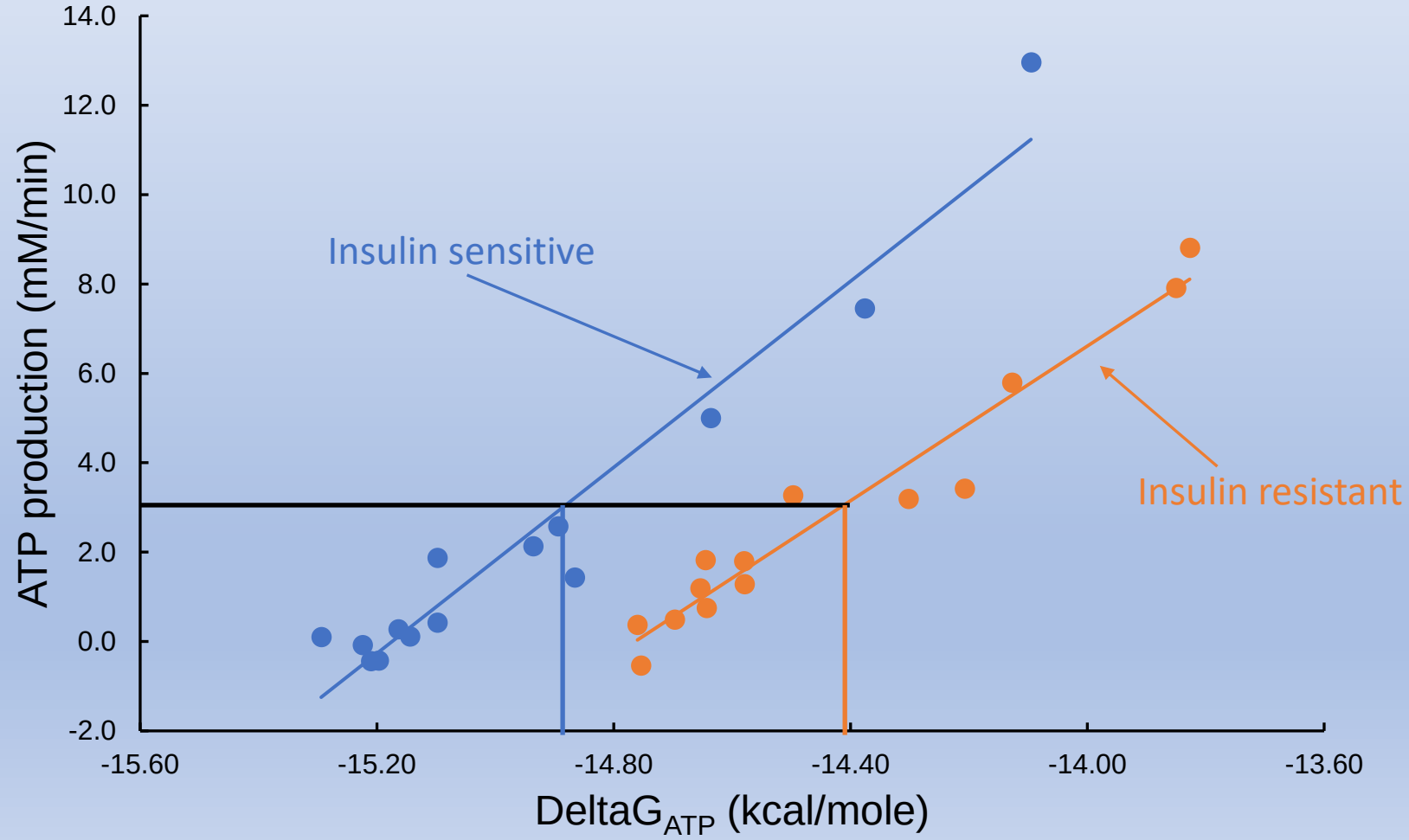
(Received January 9th, 1980)

Key words: Enzyme kinetics; Thermodynamics; Respiration; Energy-transducing system; Linear flow-force relation; (Mitochondria)

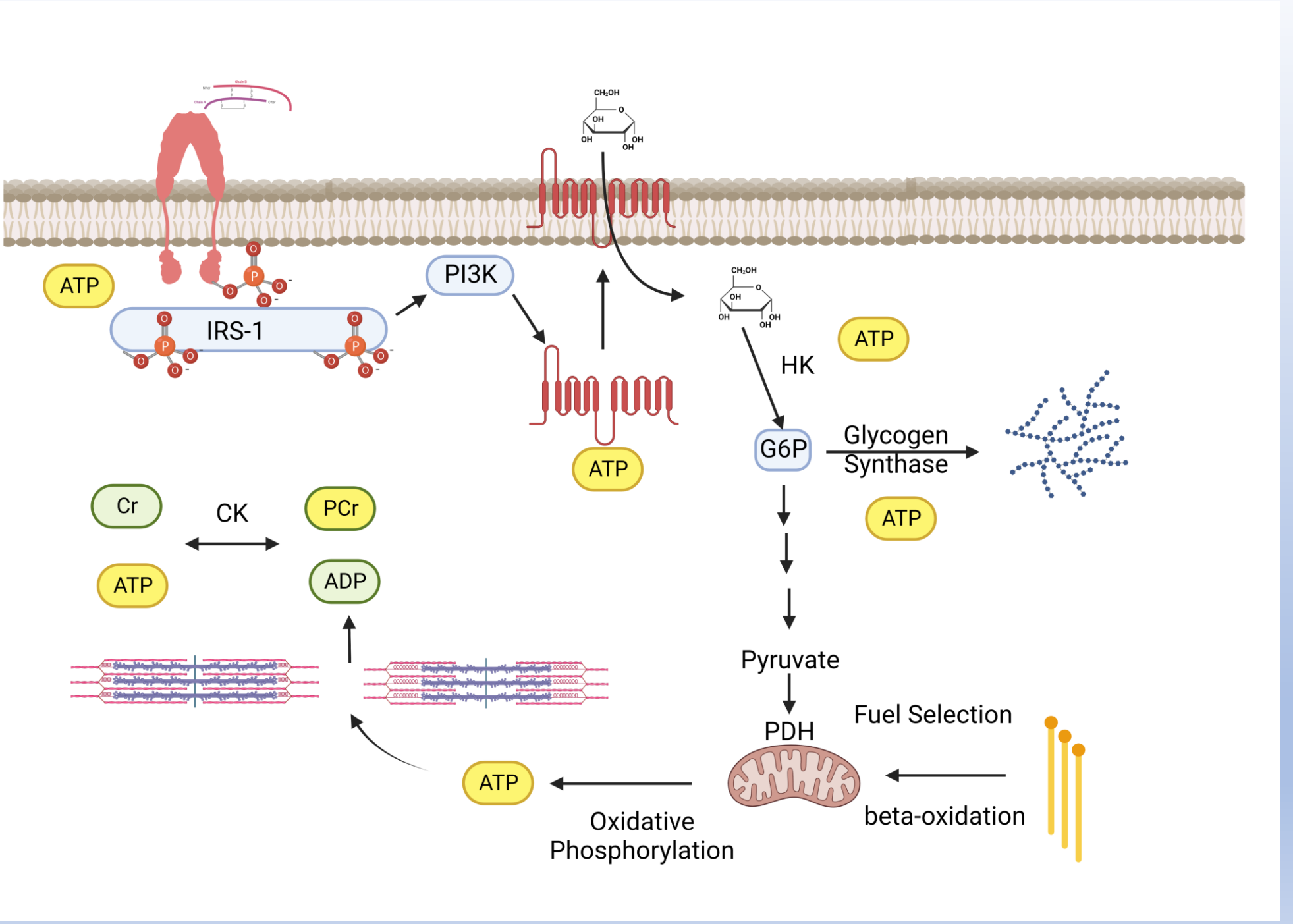
Summary

Starting from enzyme kinetics, it is shown that generally a linear rather than a proportional relationship exists between rate and free energy changes in biochemical processes. In the derivation the boundary condition of constant substrate plus product is used, which is appropriate for many cellular systems. An example is the ADP plus ATP concentration in mitochondrial oxidative phosphorylation, as is illustrated experimentally.

The energy expenditure required for insulin action results in a lower DGATP. Thus, reactions requiring ATP will proceed more slowly. This can be explained by lower mitochondrial content.



Almost all reactions related to insulin action require energy and their rates therefore depend on ΔGATP.



So, Jerry had it almost right with respect to glucose uptake and was, as usual, prescient.

Jerry's model for glucose uptake:

$V = k_u$ X [Glucose]

Where V = rate of glucose uptake, k_u = an uptake constant, and $1/k_u$ is "impedance"

From Ohm's Law, Conductance = $J_{ss}/\Delta G$, so

$J_{ss} = C \times \Delta G$

Where J_{ss} = rate of steady state glucose uptake, C = conductance, and ΔG = change in free energy due to glucose transport and phosphorylation (hexokinase) and insulin resistance to glucose uptake (R) = 1/C.

And finally, lower free energy of ATP in insulin resistant muscle can explain slower rates of any reaction induced by insulin that uses ATP.

The simplest explanation for insulin resistance is lower mitochondrial functional content due to low physical activity. The lower mitochondrial content leads directly to lower free energy of ATP.

Thank you!