Protéines alimentaires et diabète de type 2

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LES ALIMENTS FONCTIONNELS

Déclaration d'intérêts en rapport avec la présentation

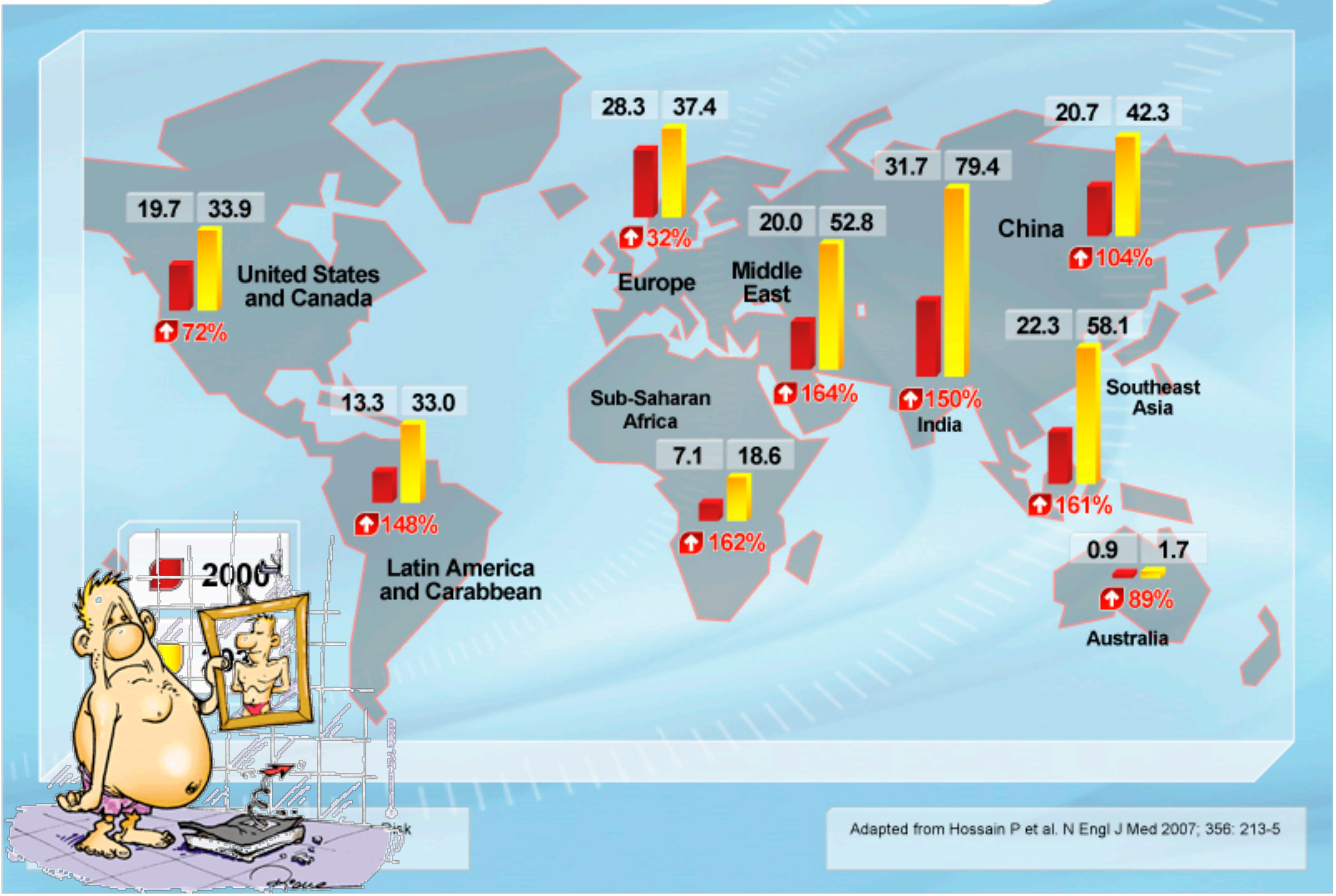
- **Activités de conseil, fonctions de gouvernance, rédaction de rapports**

  *Non / Oui **   *Société(s) : Danone, Rigel, Micropharma*

Protéines alimentaires et diabète de type 2

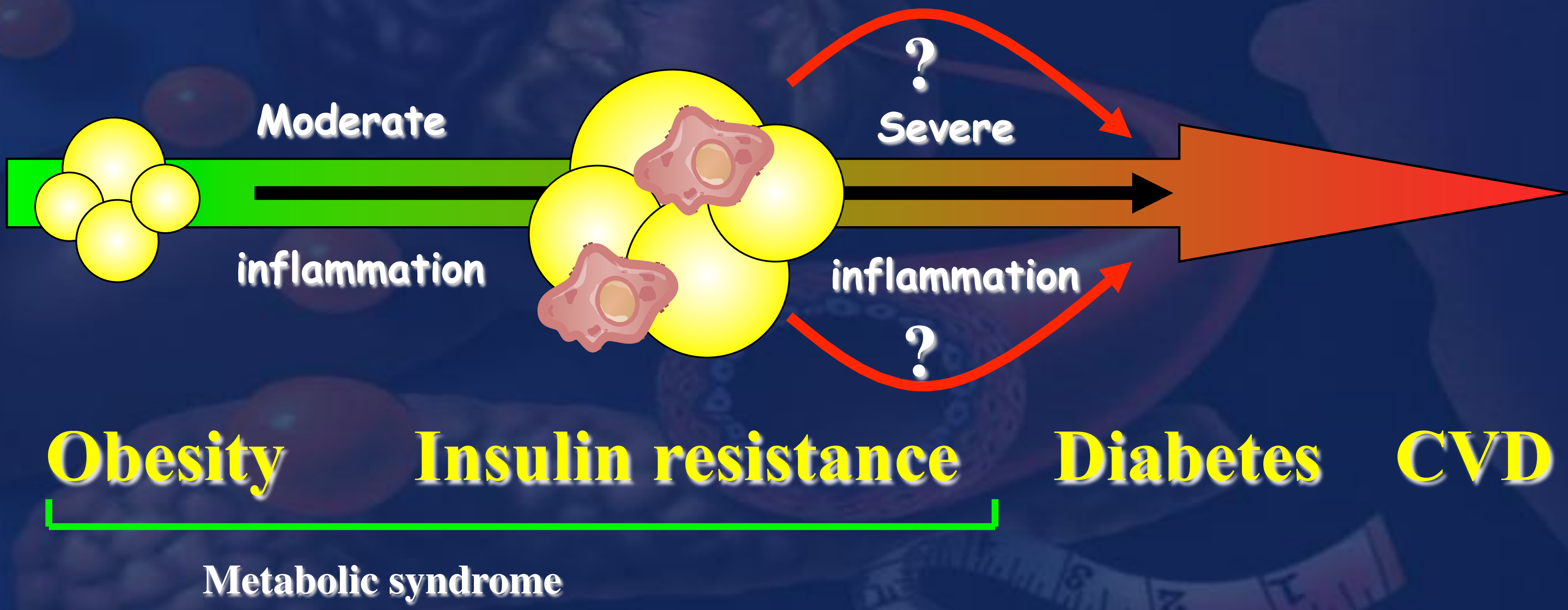
1) **Protéines alimentaires et contrôle du diabète de type 2 (DT2)**
2) **Acides aminés, insulino-résistance et DT2**
3) **BCAA dans obésité: marqueurs ou causes du DT2 ?**
4) **Peptides bioactifs et prévention du DT2**
5) **Conclusion et perspectives**

WORLDWIDE PREVALENCE OF DIABETES IN 2000 AND ESTIMATES FOR THE YEAR 2030 (IN MILLIONS)



Adapted from Hossain P et al. N Engl J Med 2007; 356: 213-5

The pathogenic continuum of obesity-linked diseases



Our “toxic” sedentary environment

Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes[1–3]

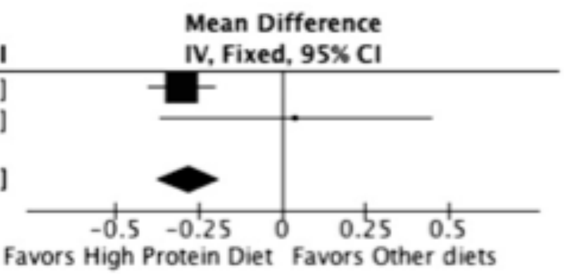
Olubukola Ajala, Patrick English, and Jonathan Pinkney

Conclusion: Low-carbohydrate, low-GI, Mediterranean, and high-protein diets are effective in improving various markers of cardiovascular risk in people with diabetes and should be considered in the overall strategy of diabetes management. *Am J Clin Nutr* 2013;97:505–16.

D Difference in Hb A_{1c} high-protein versus other diets

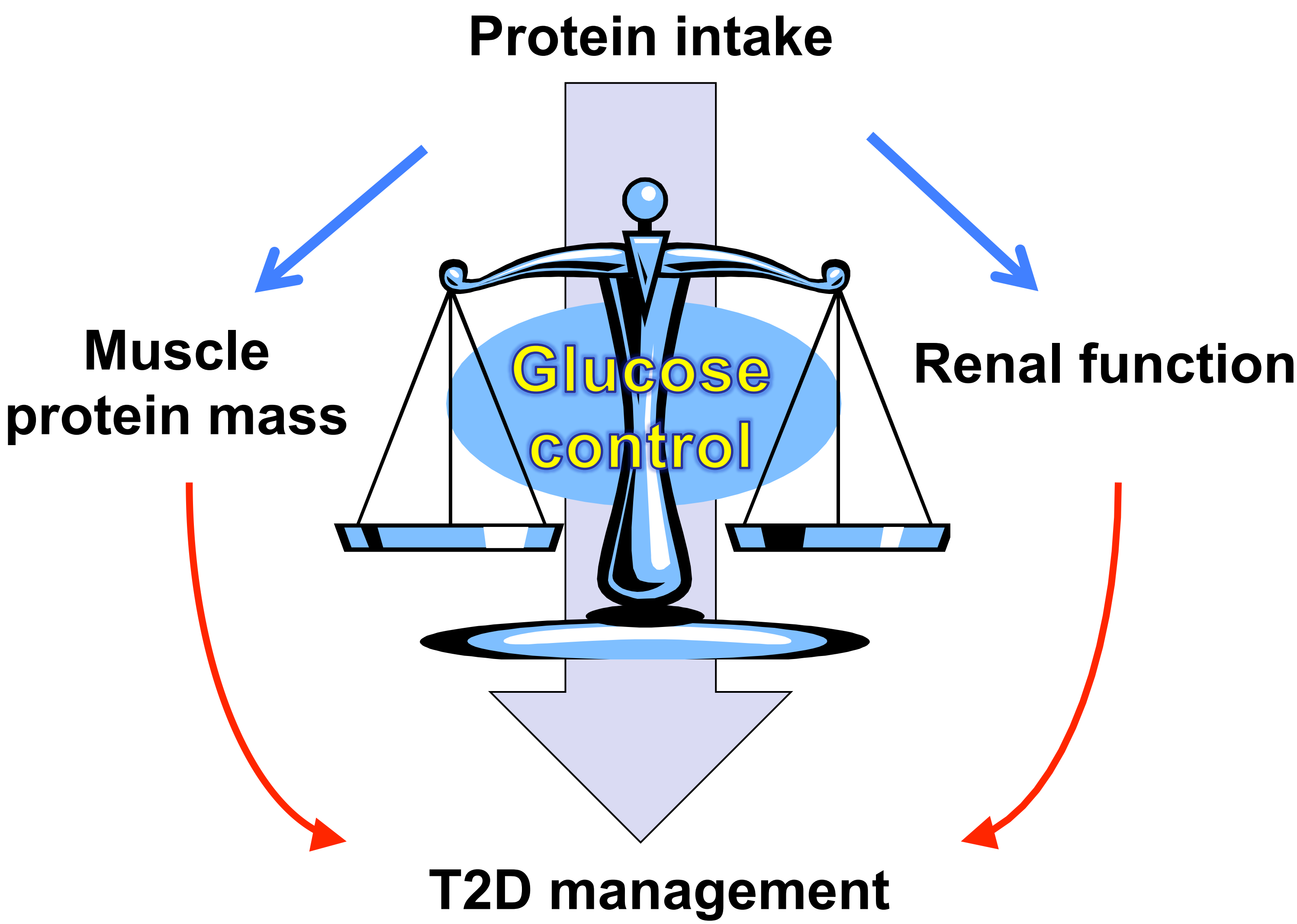
Study or Subgroup	Mean Difference	SE	High Protein Total	Other diets Total	Weight	Mean Difference IV, Fixed, 95% CI
Brinkworth 2004 [44]	-0.3	0.051	19	19	94.4%	-0.30 [-0.40, -0.20]
Larsen 2011 [40]	0.04	0.2092	53	46	5.6%	0.04 [-0.37, 0.45]
Total (95% CI)			**72**	**65**	**100.0%**	**-0.28 [-0.38, -0.18]**

Heterogeneity: $Chi^2 = 2.49$, $df = 1$ ($P = 0.11$); $I^2 = 60\%$
Test for overall effect: $Z = 5.67$ ($P < 0.00001$)



Difference in high protein vs. 'other' diets. 'Other' diets compared were low protein (Brinkworth [44]) and high carbohydrate (Larsen [40]).

Dietary protein intake in T2D subjects: a two-edges sword



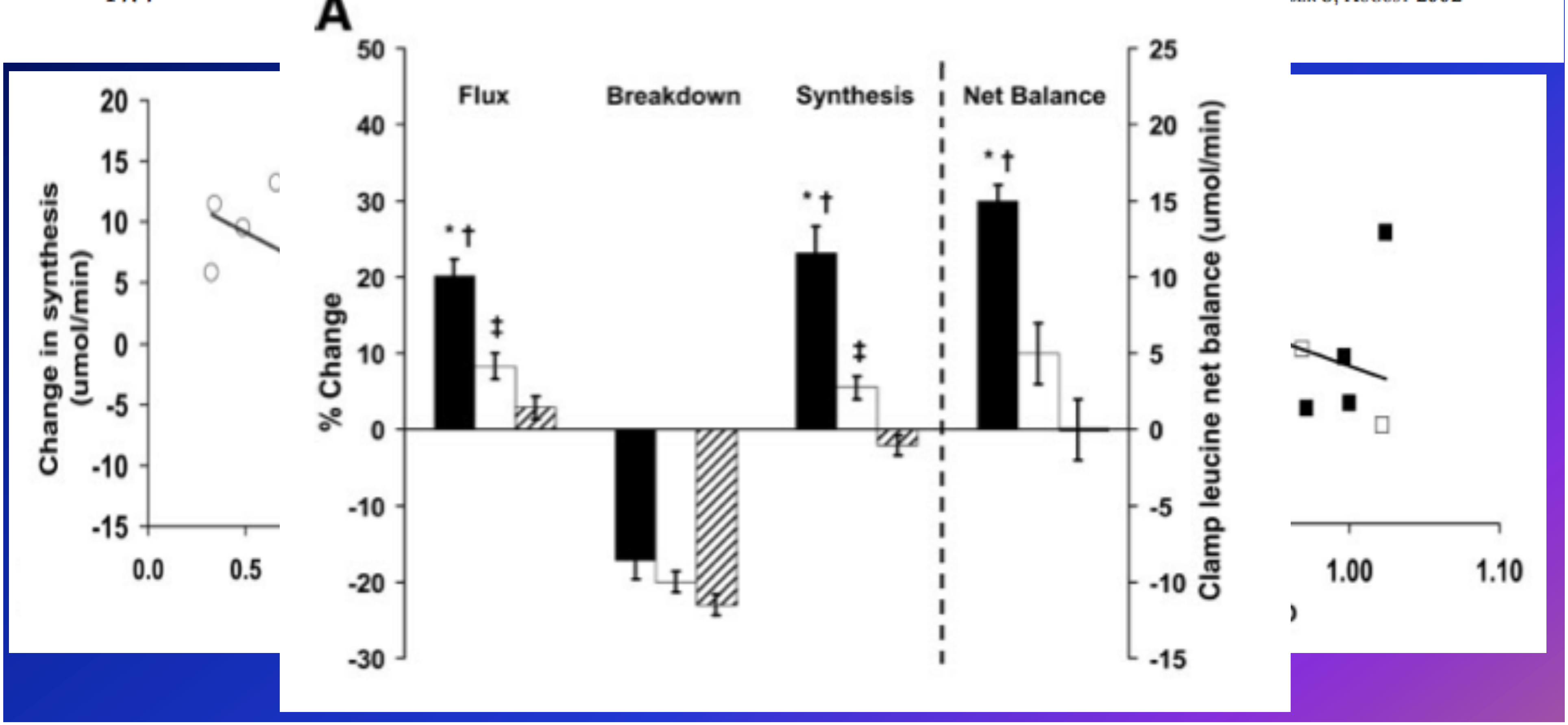
Editorials

EDITORIAL (SEE WHEELER ET AL., P. 1277)

E. Marliss and R. Gougeon

Diabetes Mellitus, Lipidus Et. . .

1474 ... BER 8, AUGUST 2002



Est-ce que la composition des protéines alimentaires peut avoir un impact sur le développement du diabète de type 2 ?

Effects of…

Obesity, diabetes and CVD		Dietary Fish
↑	insulin resistance	↓
↑	glycemia	↓
↑	inflammation	↓
↑	triglyceridemia	↓
↑	LDL-cholesterol	↓
↓	HDL-cholesterol	↑

Protocol

Male Wistar rats (200-250g)

↓

Chow or High-Fat diets (28 days)

<u>High-fat diet</u>

- 40% Fat (20% Lard, 20% Corn Oil)
- 27% Carbohydrate (Sucrose)
- 20% Protein (casein, cod, or soy)

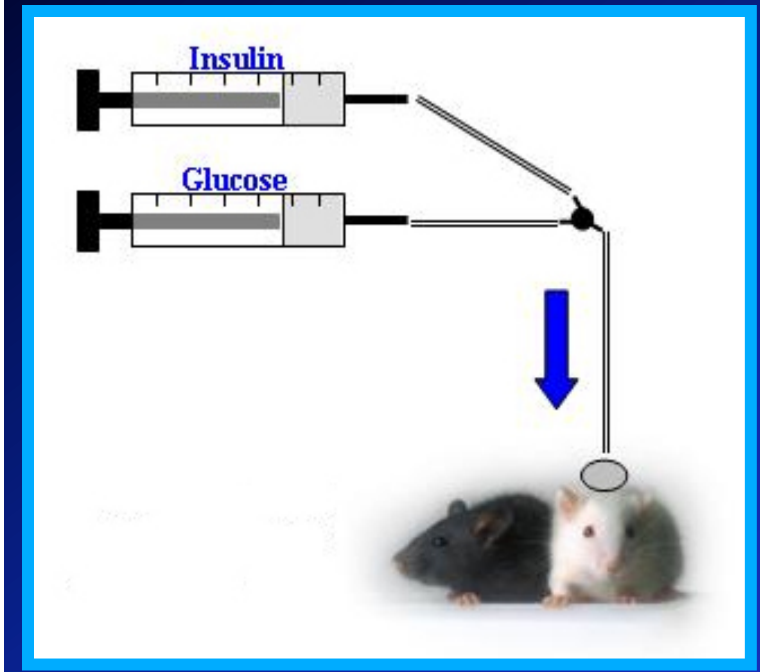
↓

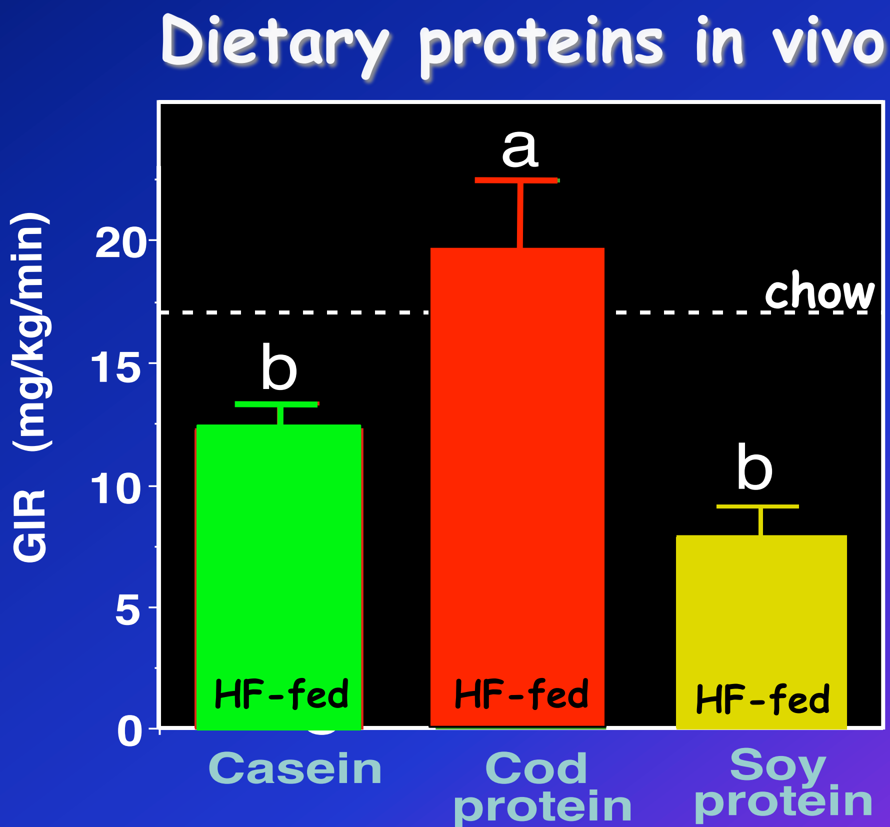
Obesity and insulin resistance

- body weight gain and adiposity
- whole-body insulin action (clamp)
- tissue insulin action (2-DG uptake)
- insulin receptor signaling

Dietary cod protein prevents insulin resistance

HIE clamp technique

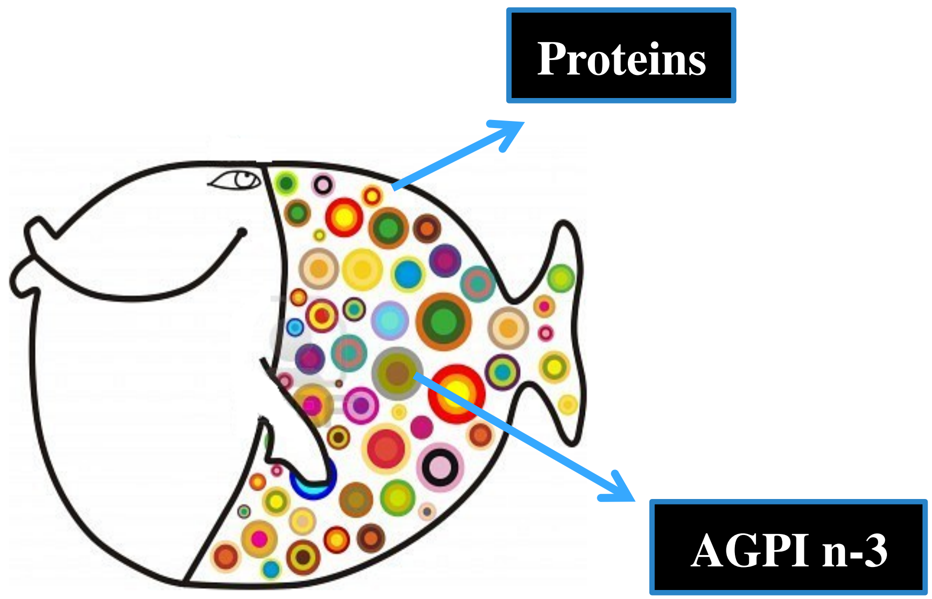




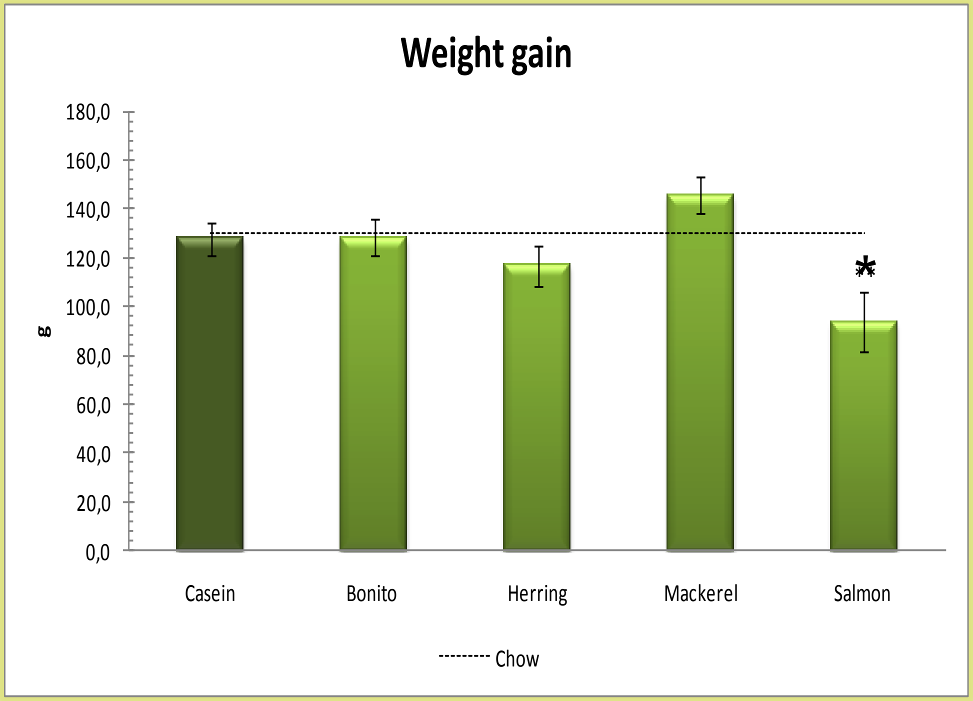
However, feeding cod protein did not affect weight gain or adiposity

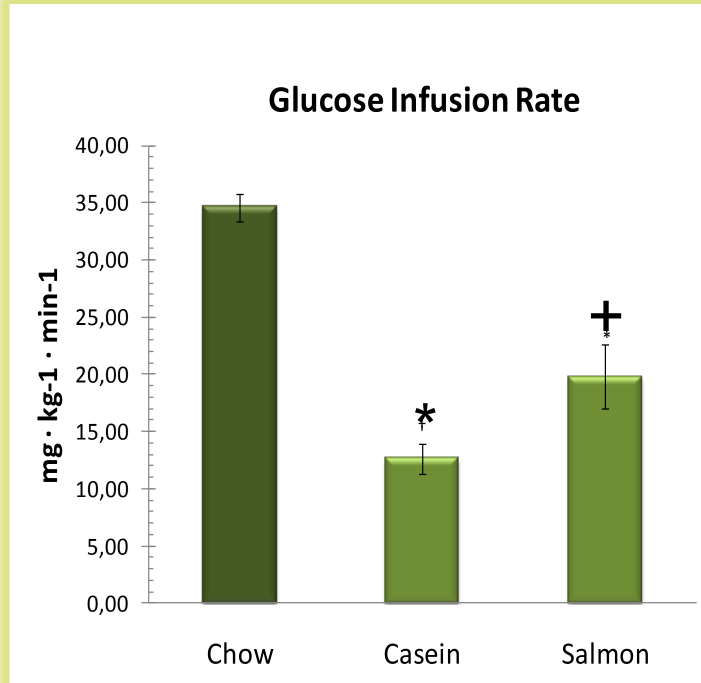
Lavigne et al., *Am. J. Physiol. 278 (Endocrinol. Metab.),* E491-E500, 2000.

Are all fish equals ?



Salmon protein hydrolysate prevents weight gain and improves insulin sensitivity in fat-fed rats





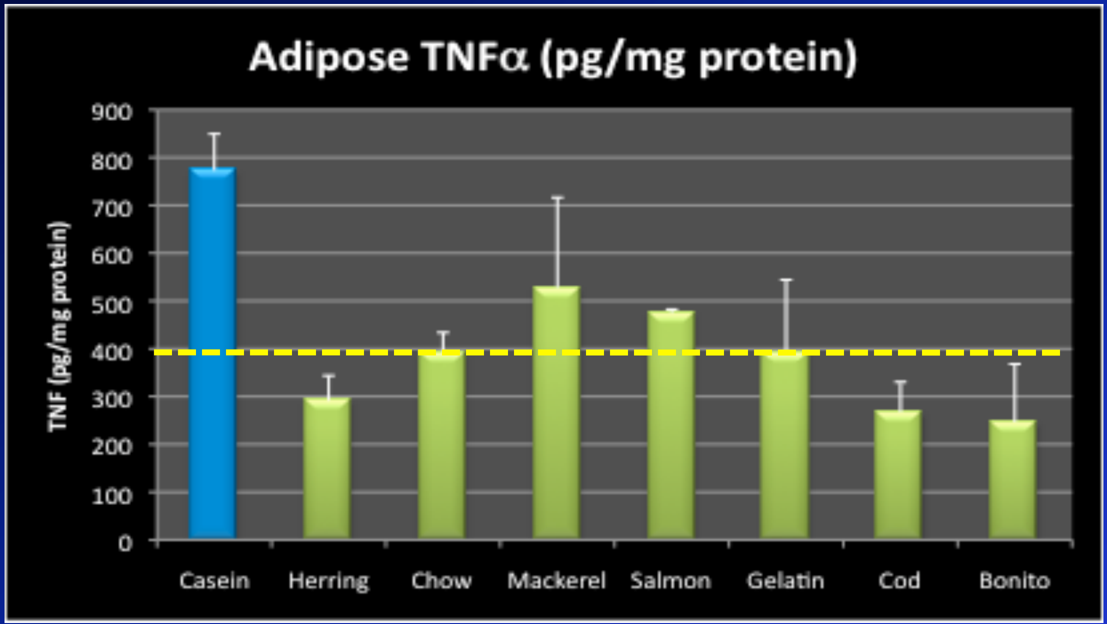
afmnet

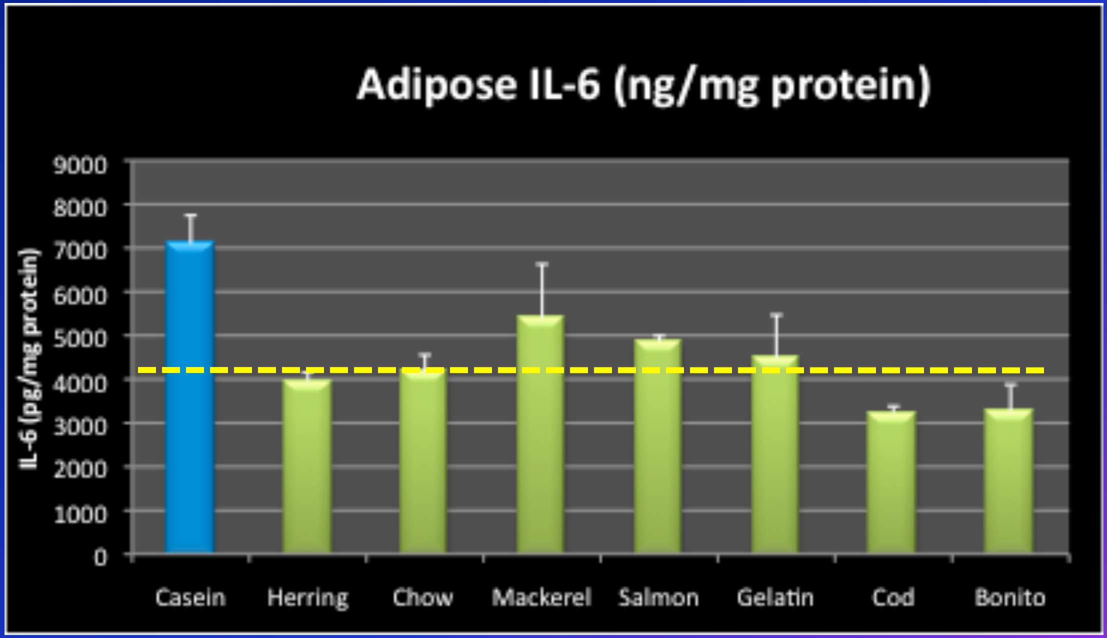
Réseau des aliments et des matériaux d'avant-garde

ADVANCED FOODS & MATERIALS NETWORK

Inspiration from the ground up | Aux racines de l'inspiration

Multiple fish protein sources carry anti-inflammatory properties





Experimental protocol in human obese subjects

Crossover design

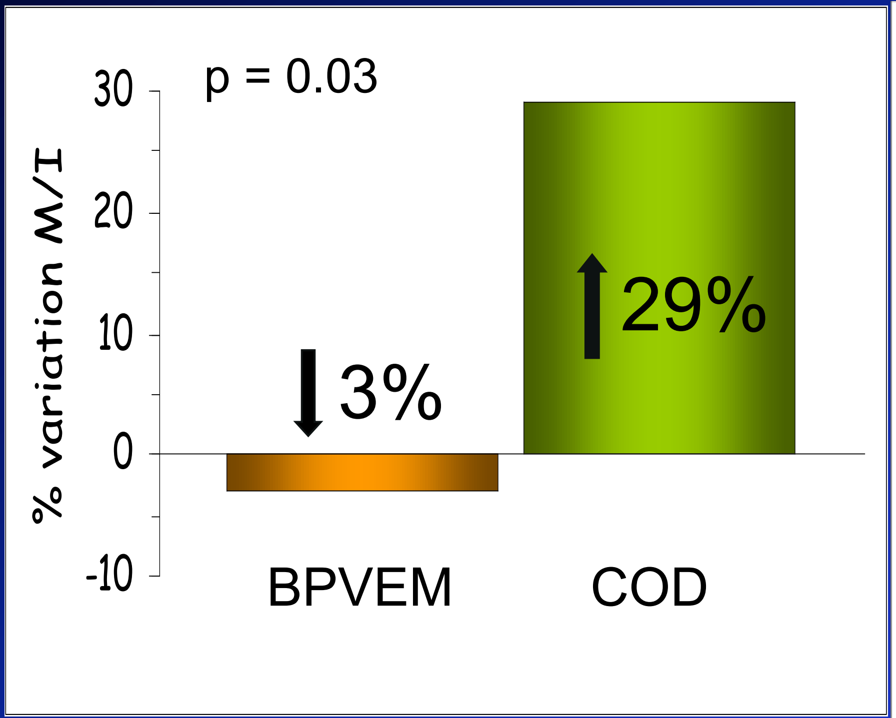
Legend

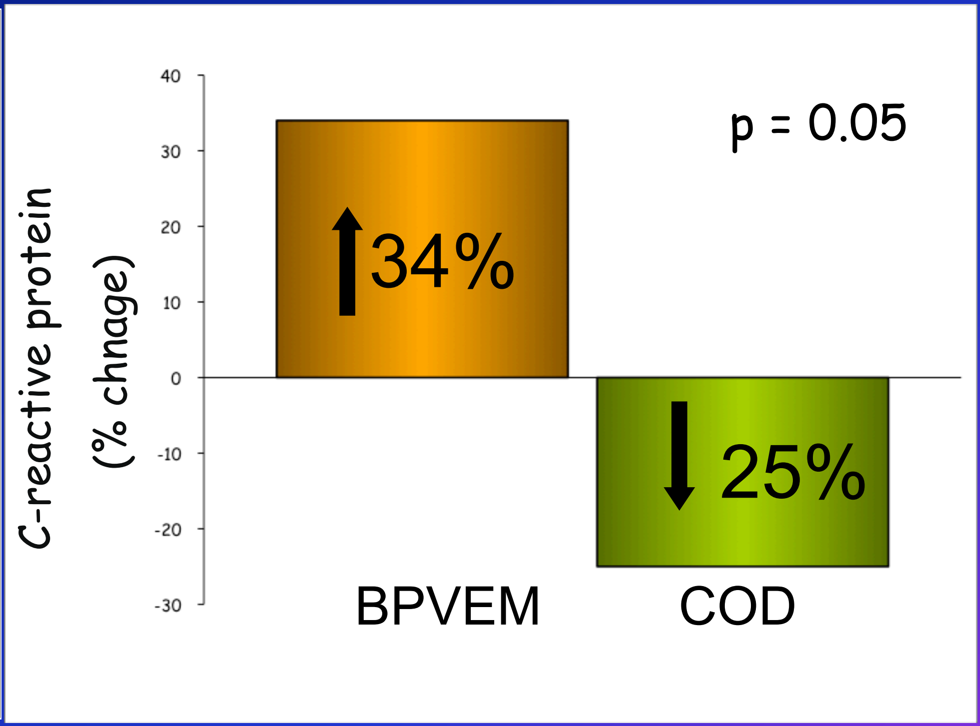
= Blood samples, OGTT, Clamp

= Biopsy

Weeks	- 2		0		4		8		12
			Blood samples		Biopsy + Blood samples		Blood samples		Biopsy + Blood samples
group 1		Run-in period	→	COD	→	washout	→	BPVEM	
group 2		Run-in period	→	BPVEM	→	washout	→	COD	

Dietary cod protein improves insulin sensitivity and reduces inflammation in insulin-resistant human subjects





Ouellet et al. Diabetes Care 30: 2816-2821, 2007
Ouellet et al. J Nutr. 138(12):2386-91, 2008

Protéines alimentaires et diabète de type 2

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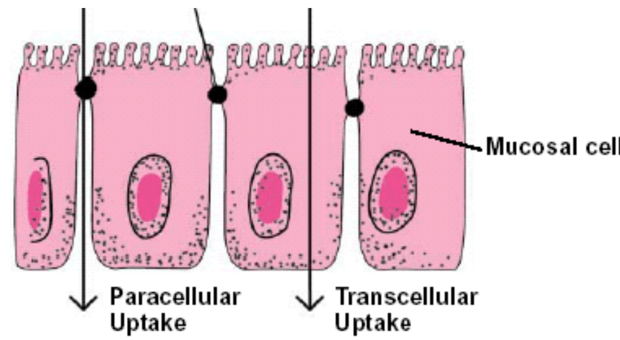
Two potential mechanisms underlying the beneficial effects of fish proteins on T2D

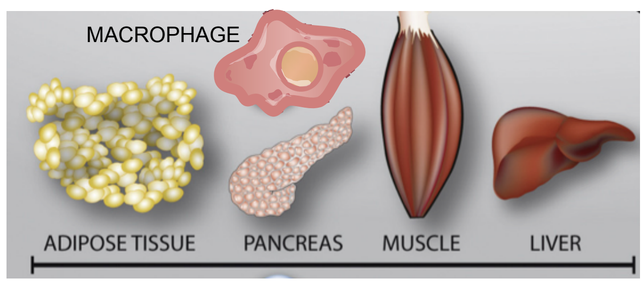
Fish proteins

biopeptides **Amino acids**

effect on the gut and the microbiota



effect on metabolic tissues and immune cells



Prevention of T2D

Plasma amino acid composition of HF-fed rats consuming different dietary proteins

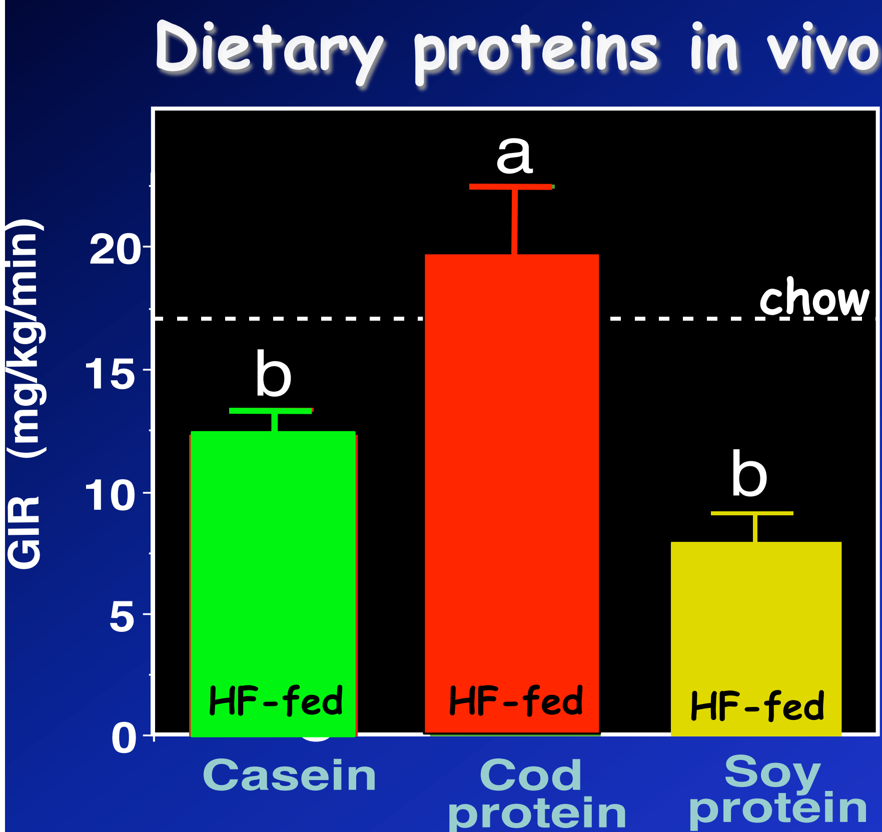


Table 1. *Dietary protein-derived amino acid mixtures used for L6 culture conditions*

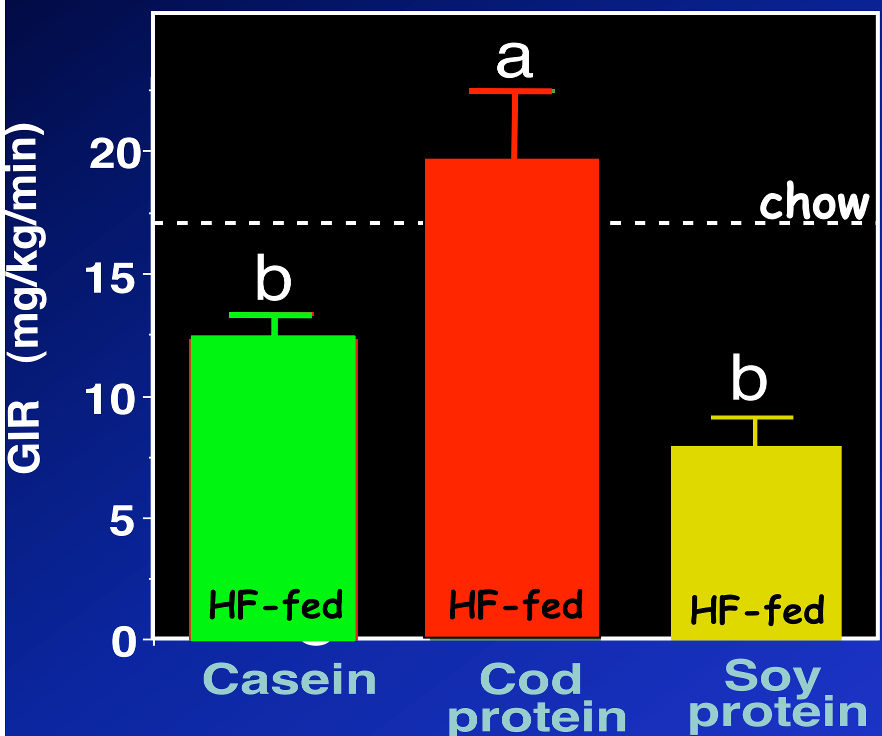
	Casein	Cod Protein	Soy Protein	Chow
L-Alanine	663	563	501	603
L-Arginine	150	194	176	137
L-Asparagine	129	111	121	108
L-Asparctic acid	26	21	20	19
L-Cysteine	23	29	24	15
L-Glutamic acid	110	114	211	104
L-Glutamine	1263	925	1027	1370
L-Glycine	215	255	272	382
L-Histidine	76	67	68	58
L-Isoleucine	115	90	98	89
L-Leucine	170	122	130	131
L-Lysine	470	441	386	373
L-Methionine	98	102	67	76
L-Phenylalanine	104	76	82	76
L-Proline	366	189	222	285
L-Serine	291	218	270	283
L-Threonine	366	261	277	386
L-Tyrosine	112	66	85	91
L-Valine	230	163	166	176

Amino acid mixtures are expressed in μM.

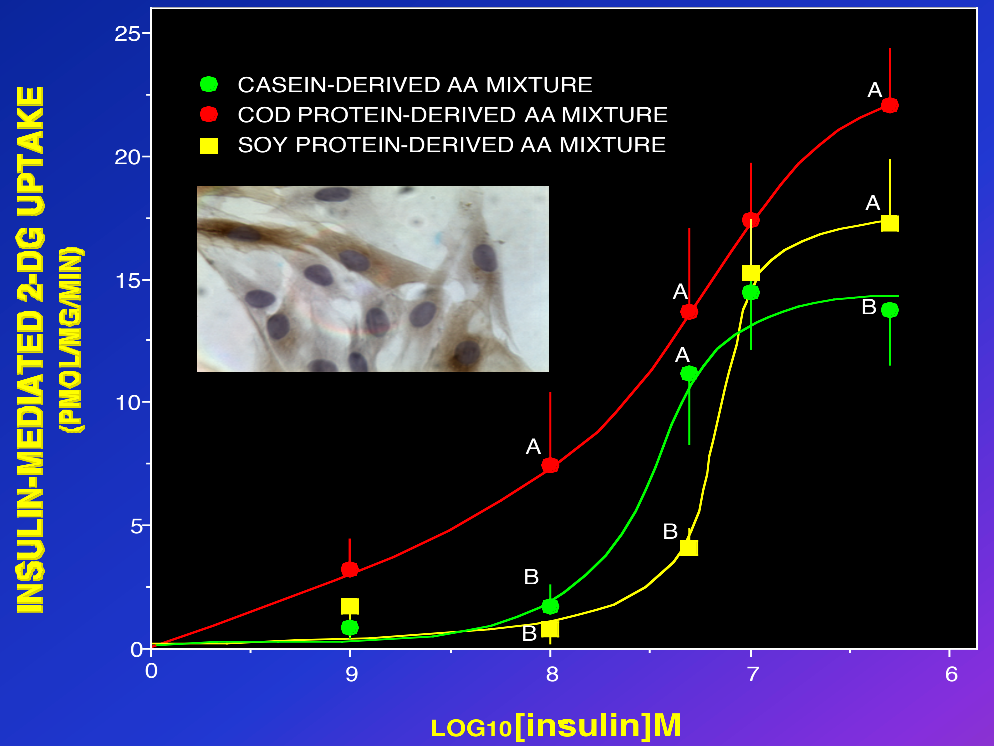
Lavigne et al. Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. *Am J Physiol Endocrinol Metab*. 2001 Jul;281 (1):E62-71.

The effects of dietary proteins can be reproduced by amino acids *in vitro*

Dietary proteins in vivo

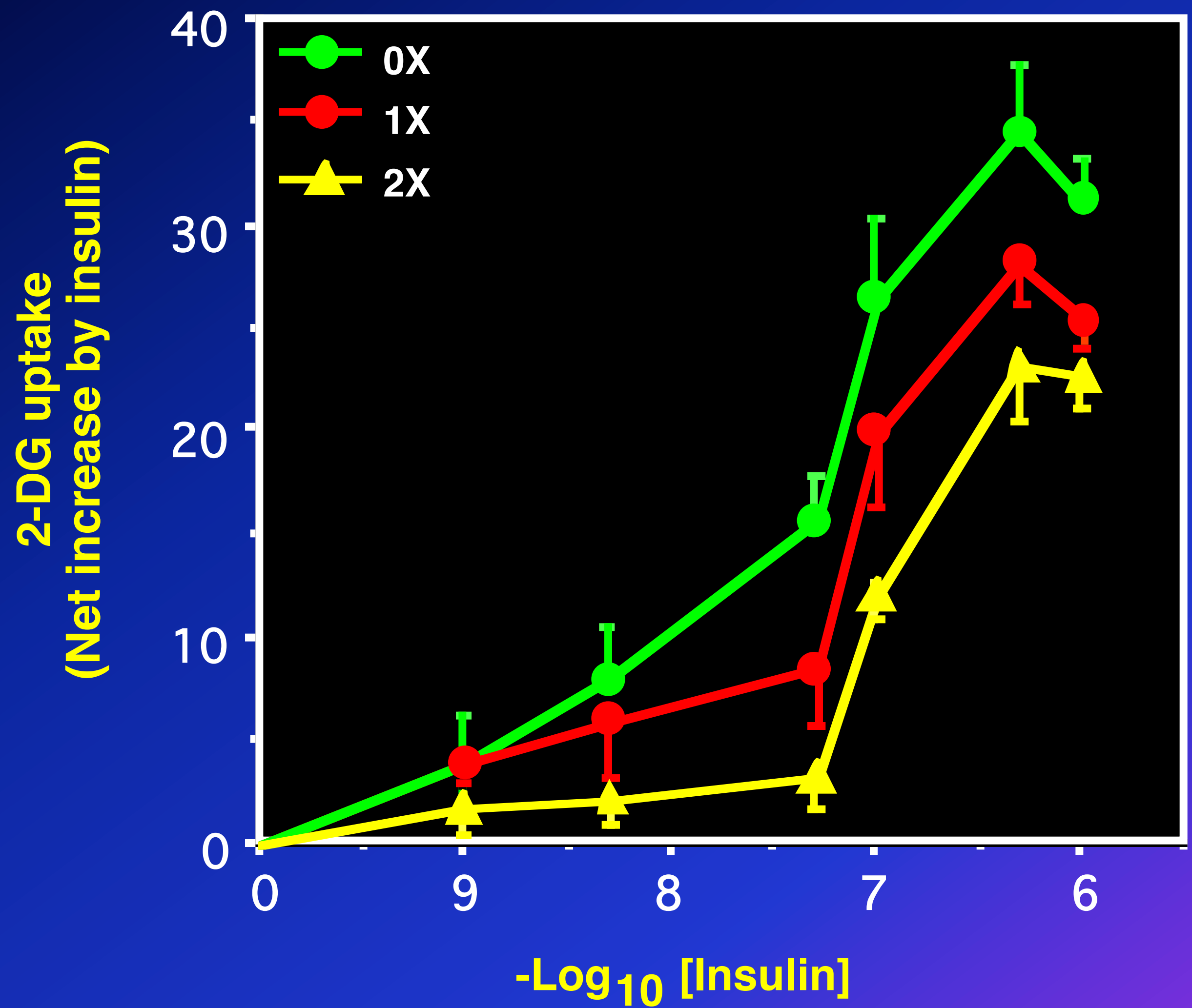


Amino acids in vitro



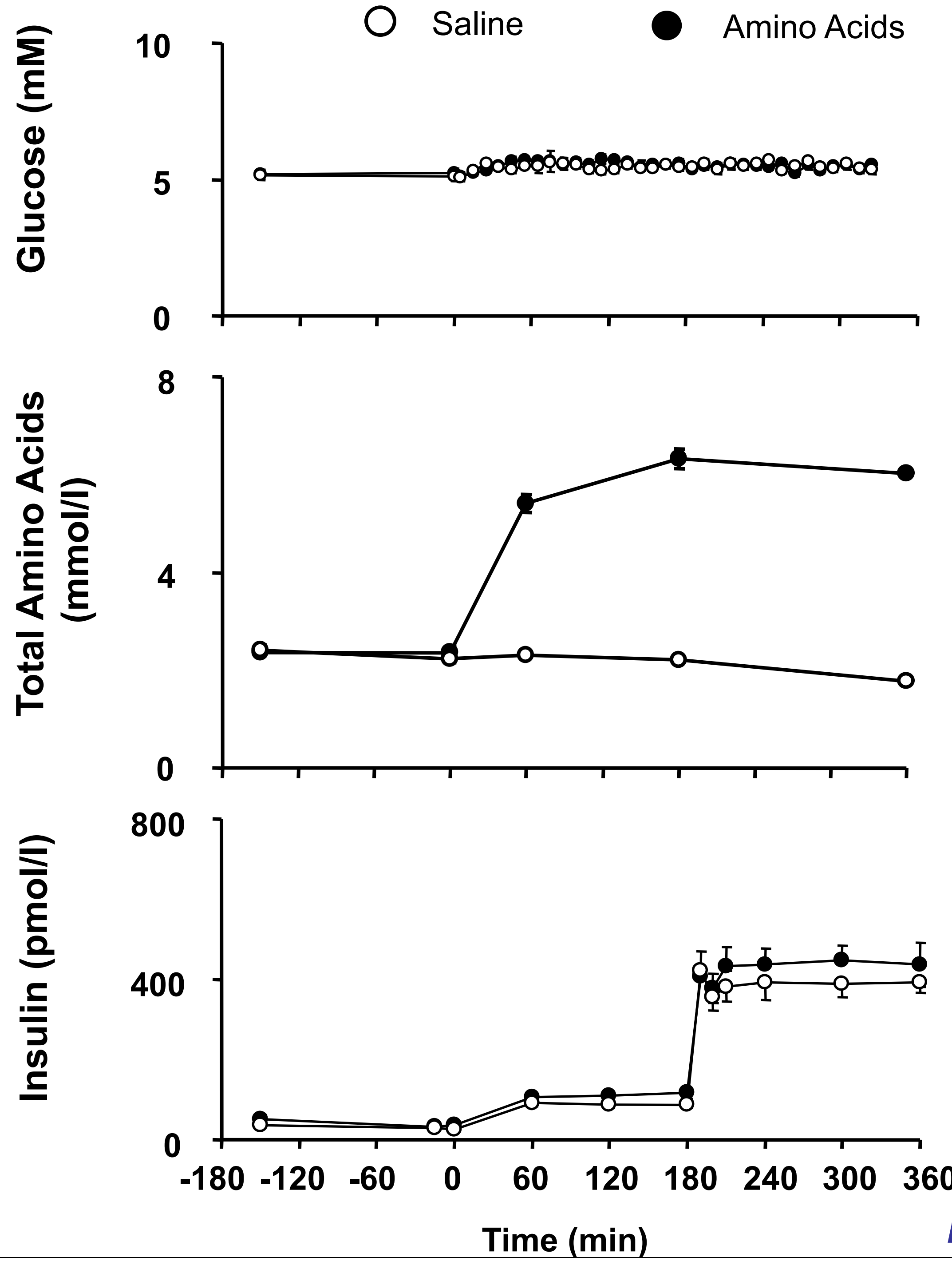
Lavigne et al. Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. *Am J Physiol Endocrinol Metab*. 2001 Jul;281 (1):E62-71.

Amino acids inhibit insulin-stimulated glucose uptake in L6 myocytes



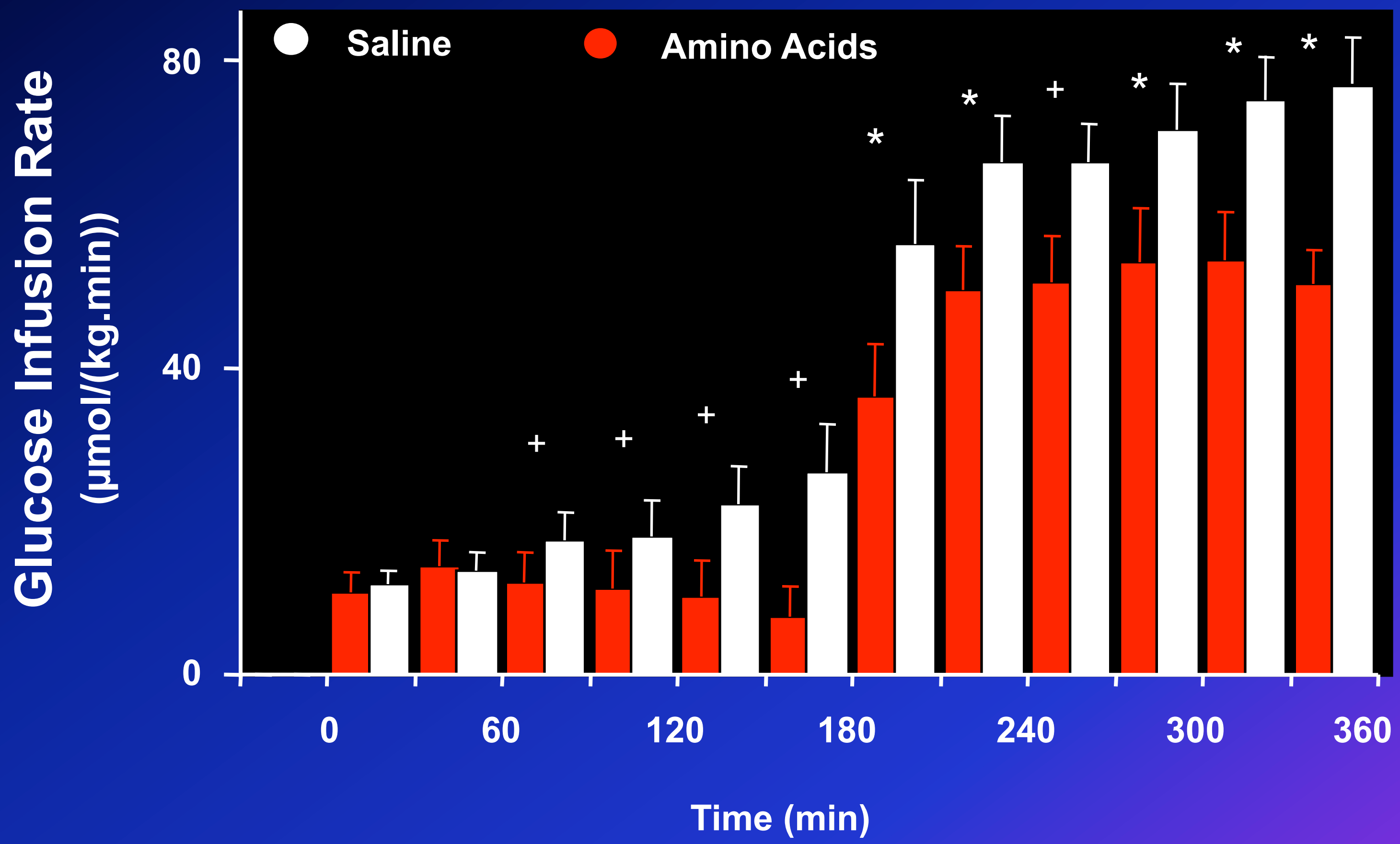
J Biol Chem (2001) 276:38052

Is mTORC1/S6K1-mediated insulin resistance relevant to human insulin resistance ?



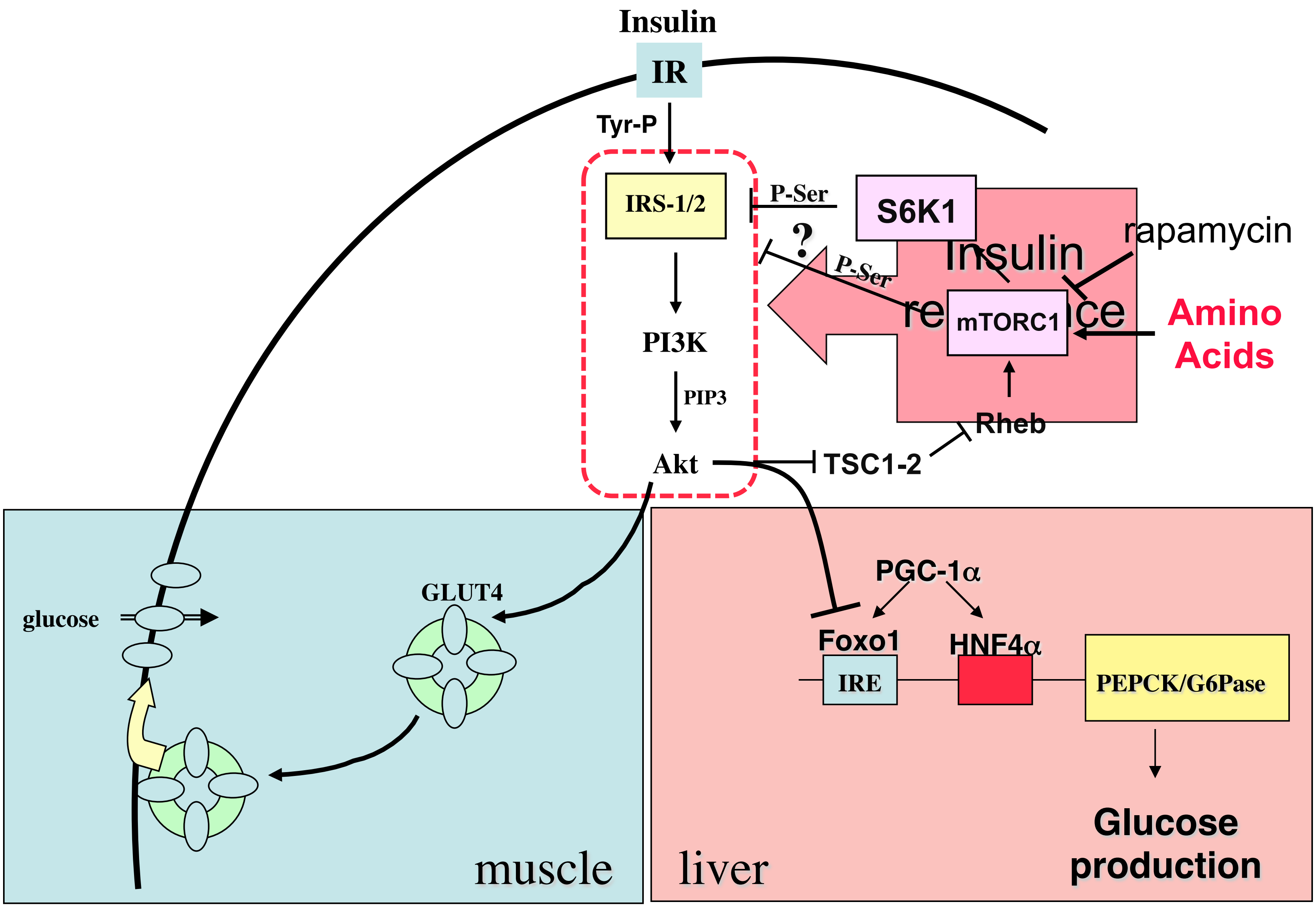
Diabetes 2005 Sep;54(9):2674-8

Amino acid infusion impairs insulin-stimulated glucose uptake in humans

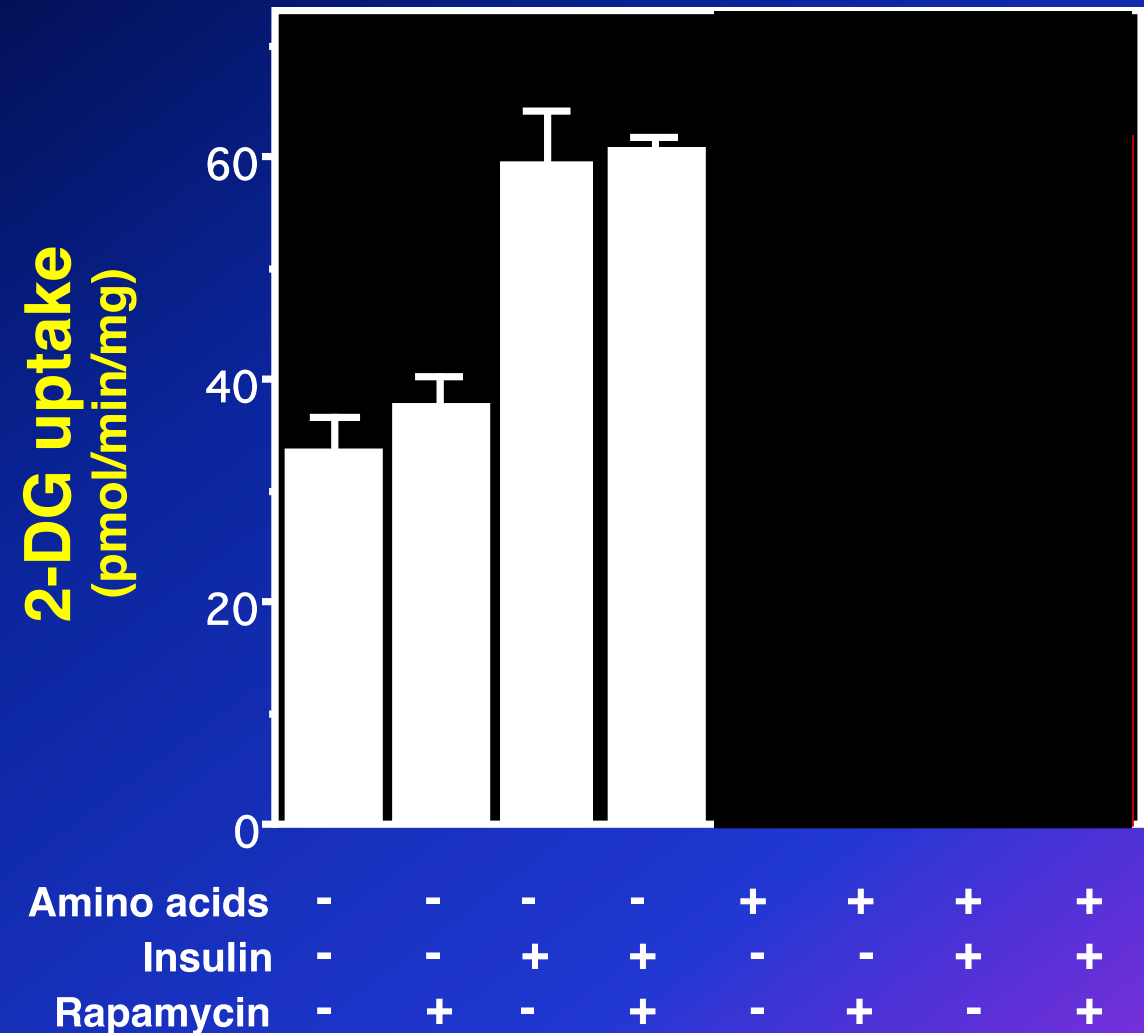


Diabetes 2005 Sep;54(9):2674-8

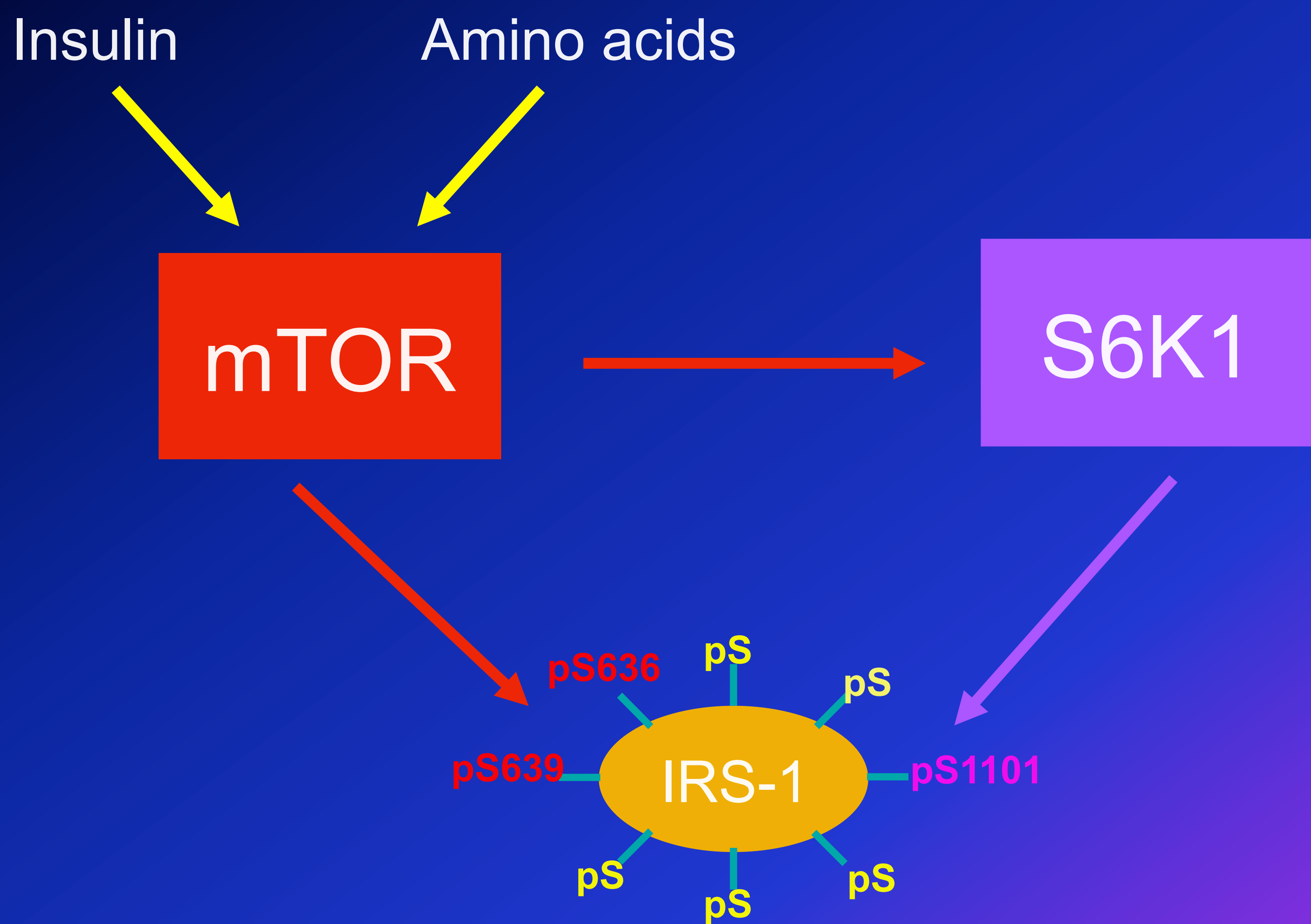
Integrative view of molecular pathways implicated in the pathogenesis of muscle and liver insulin resistance through activation of iNOS



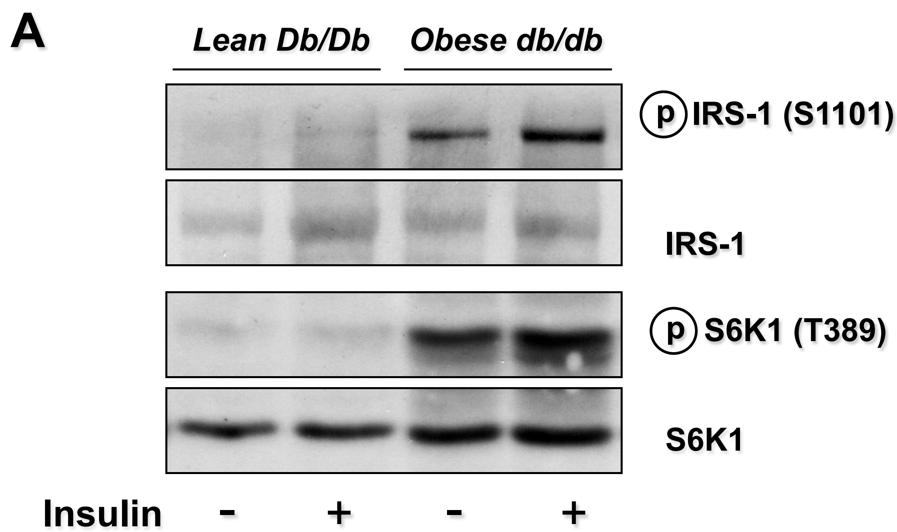
Rapamycin reverses the inhibitory effects of amino acids on insulin-induced glucose uptake



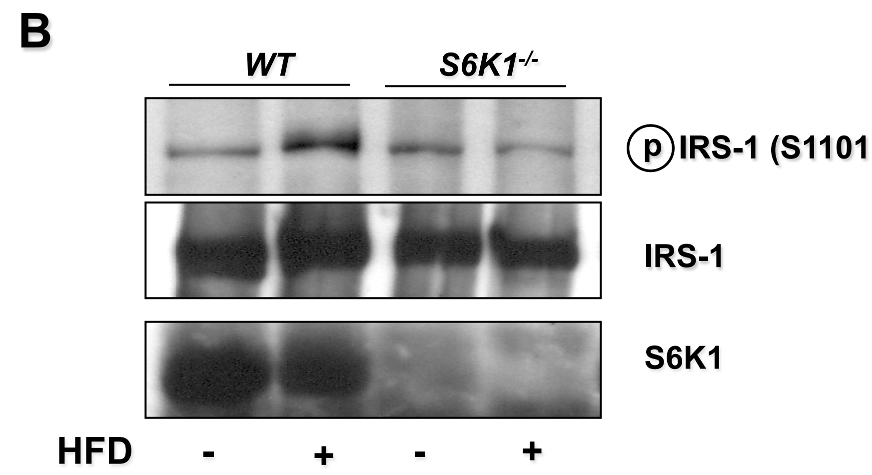
mTOR and S6K1 phosphorylate distinct serine residues

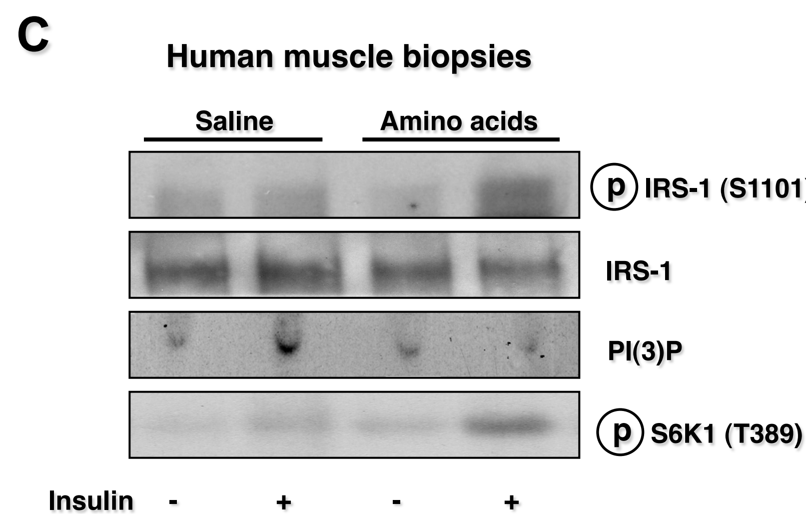


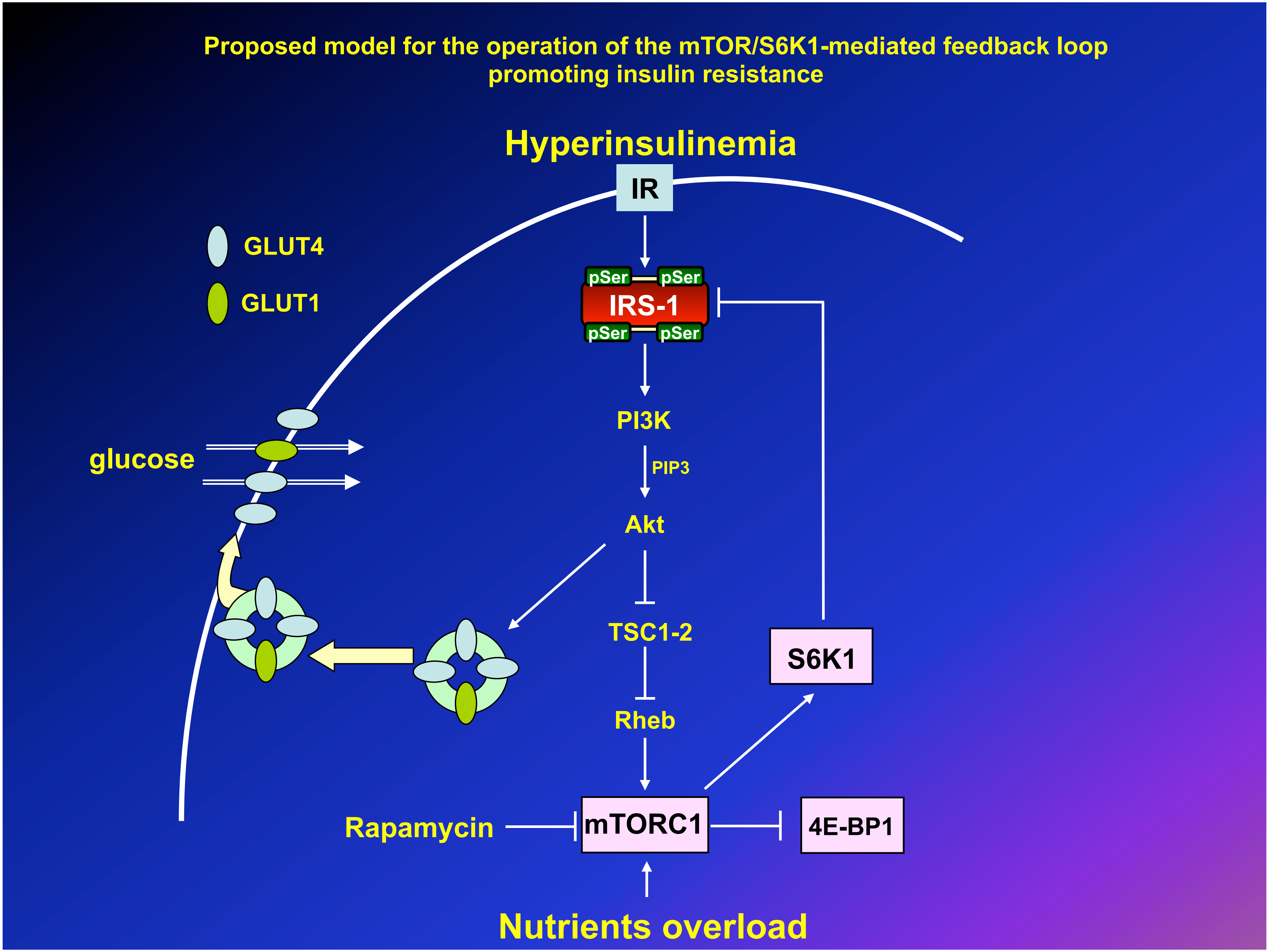
Overactivation of S6K1 in obesity increases IRS-1 S1101 phosphorylation



Genetic disruption of S6K1 blunts IRS-1 S1101 phosphorylation in mice and cells






Proposed model for the operation of the mTOR/S6K1-mediated feedback loop
promoting insulin resistance
Hyperinsulinemia
IR
GLUT4
GLUT1
pSer
pSer
IRS-1
pSer
pSer
PI3K
PIP3
Akt
glucose
TSC1-2
S6K1
Rheb
Rapamycin
mTORC1
4E-BP1
Nutrients overload

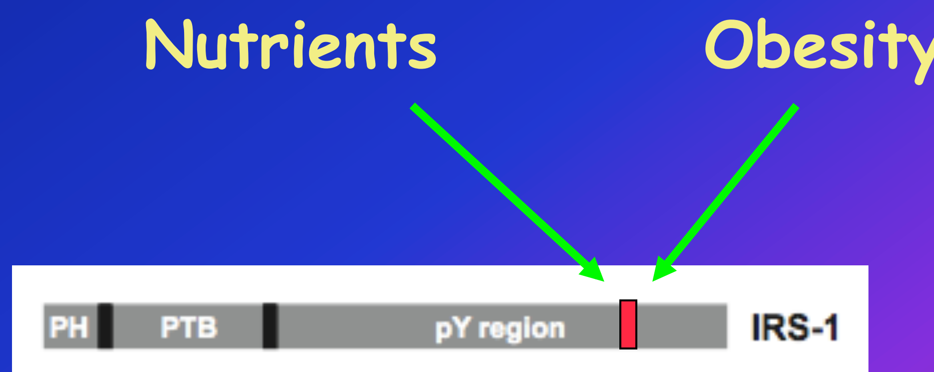
PNAS August 28, 2007 vol. 104 no. 35, 14056–14061

Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance

Frédéric Tremblay[*,†], Sophie Brûlé[*], Sung Hee Um[‡], Yu Li[§], Kohei Masuda[‡], Michael Roden[¶], Xiao Jian Sun[‖], Michael Krebs[**], Roberto D. Polakiewicz[§], George Thomas[‡‡‡], and André Marette[*,††]

[*]Department of Anatomy and Physiology and Lipid Research Unit, Laval University Hospital Research Center, Ste-Foy, QC, Canada G1V 4G2; [‡]Genome Research Institute, University of Cincinnati, Cincinnati, OH 45237; [§]Cell Signaling Technology, Beverly, MA 01923; [¶]Medical Department, Hanusch Hospital, Heinrich Collin Strasse 30, A-1140 Vienna, Austria; [‖]Section of Endocrinology, University of Chicago, Chicago, IL 60637; and [**]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, A-1010 Vienna, Austria

Frédéric Tremblay   Sophie Brûlé



AAs…a two-edges sword ?

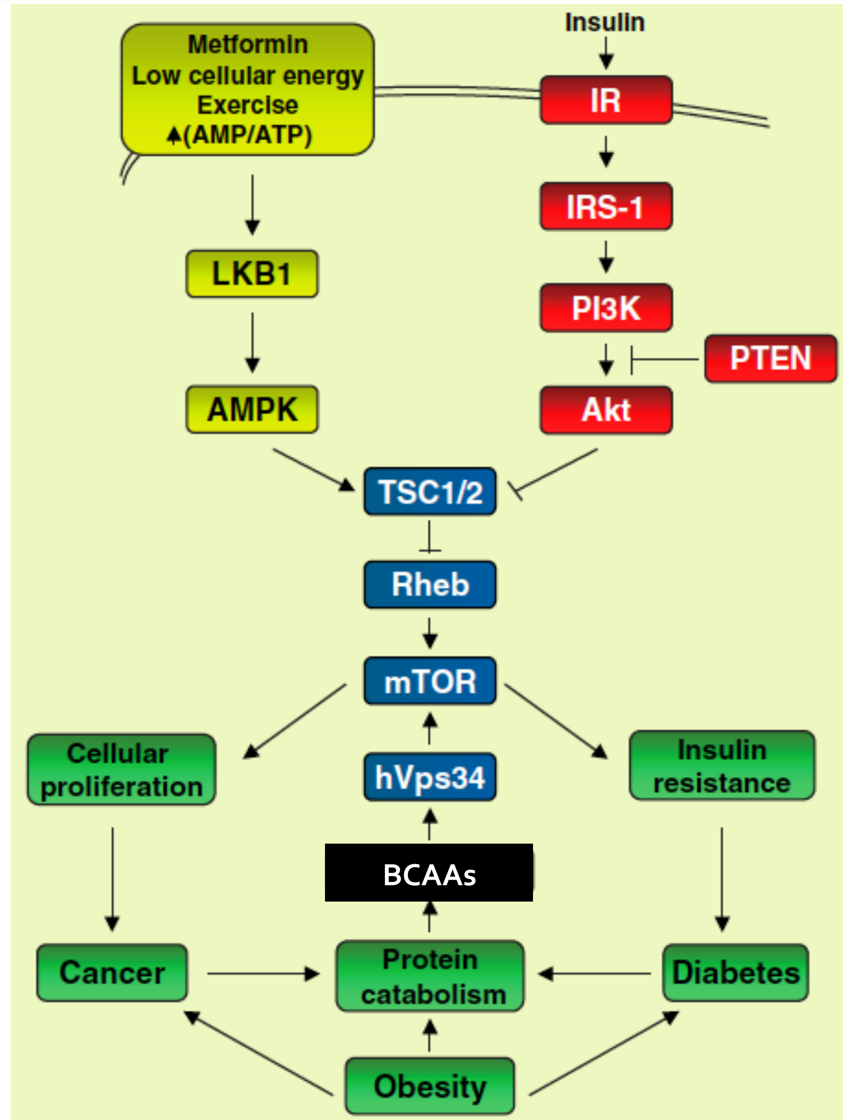
Annu. Rev. Nutr. 2007. 27:293–310

First published online as a Review in Advance on April 27, 2007

Role of Dietary Proteins and Amino Acids in the Pathogenesis of Insulin Resistance

Frédéric Tremblay,[1] Charles Lavigne,[2] Hélène Jacques,[2,3] and André Marette[1,2]

[1]Department of Anatomy & Physiology and Lipid Research Unit, Laval University Hospital Research Center, Québec, Canada, [2]Institute of Nutraceuticals and Functional Foods, and [3]Department of Food Science and Nutrition, Laval University, Québec, Canada; email: andre.marette@crchul.ulaval.ca



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Cell Metabolism
Article

2009

Cell PRESS

A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance

Christopher B. Newgard,[1,2,3,*] Jie An,[1,3] James R. Bain,[1] Michael J. Muehlbauer,[1] Robert D. Stevens,[1] Lillian F. Lien,[1,2] Andrea M. Haqq,[1,4] Svati H. Shah,[2] Michelle Arlotto,[1] Cris A. Slentz,[2] James Rochon,[5] Dianne Gallup,[5] Olga Ilkayeva,[1] Brett R. Wenner,[1] William S. Yancy, Jr.,[2] Howard Eisenson,[6] Gerald Musante,[2] Richard S. Surwit,[7] David S. Millington,[1,4] Mark D. Butler,[1] and Laura P. Svetkey[1,2]

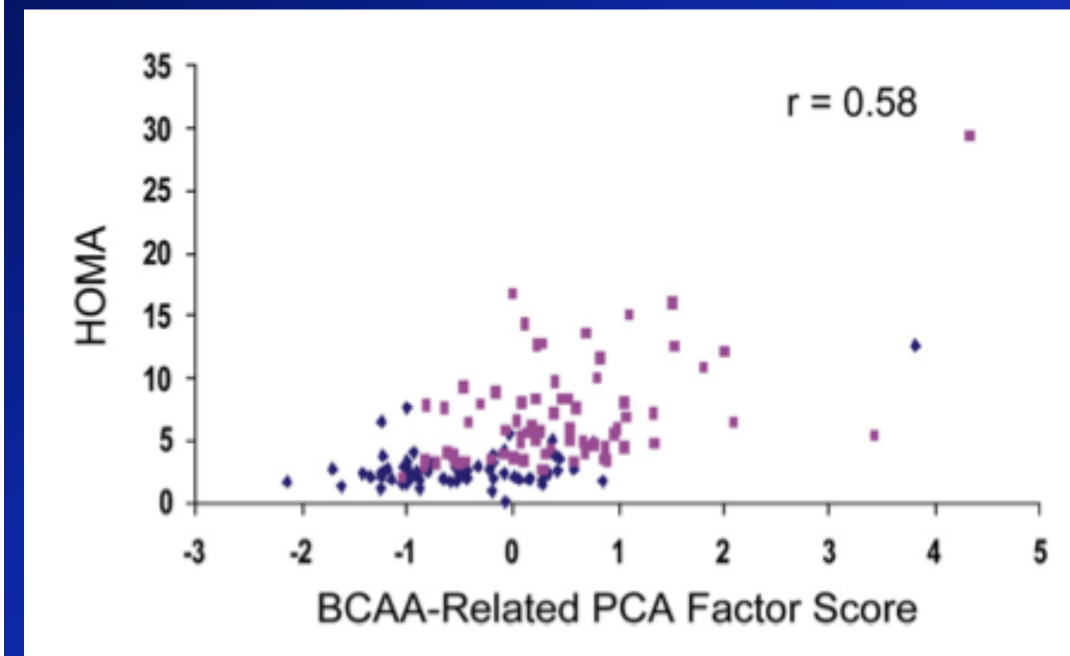


Figure 1. A BCAA-Related Metabolic "Signature" Correlates with Insulin Sensitivity
The figure shows the relationship between insulin sensitivity (HOMA) and a principal component comprised of BCAA-related metabolites, including the BCAAs valine, leucine, and isoleucine; Glx (glutamate and glutamine); the aromatic amino acids phenylalanine and tyrosine; and C3 and C5 acylcarnitines (See Experimental Procedures and Results for further description). Diamonds, lean subjects (n = 67); squares, obese subjects (n = 74).

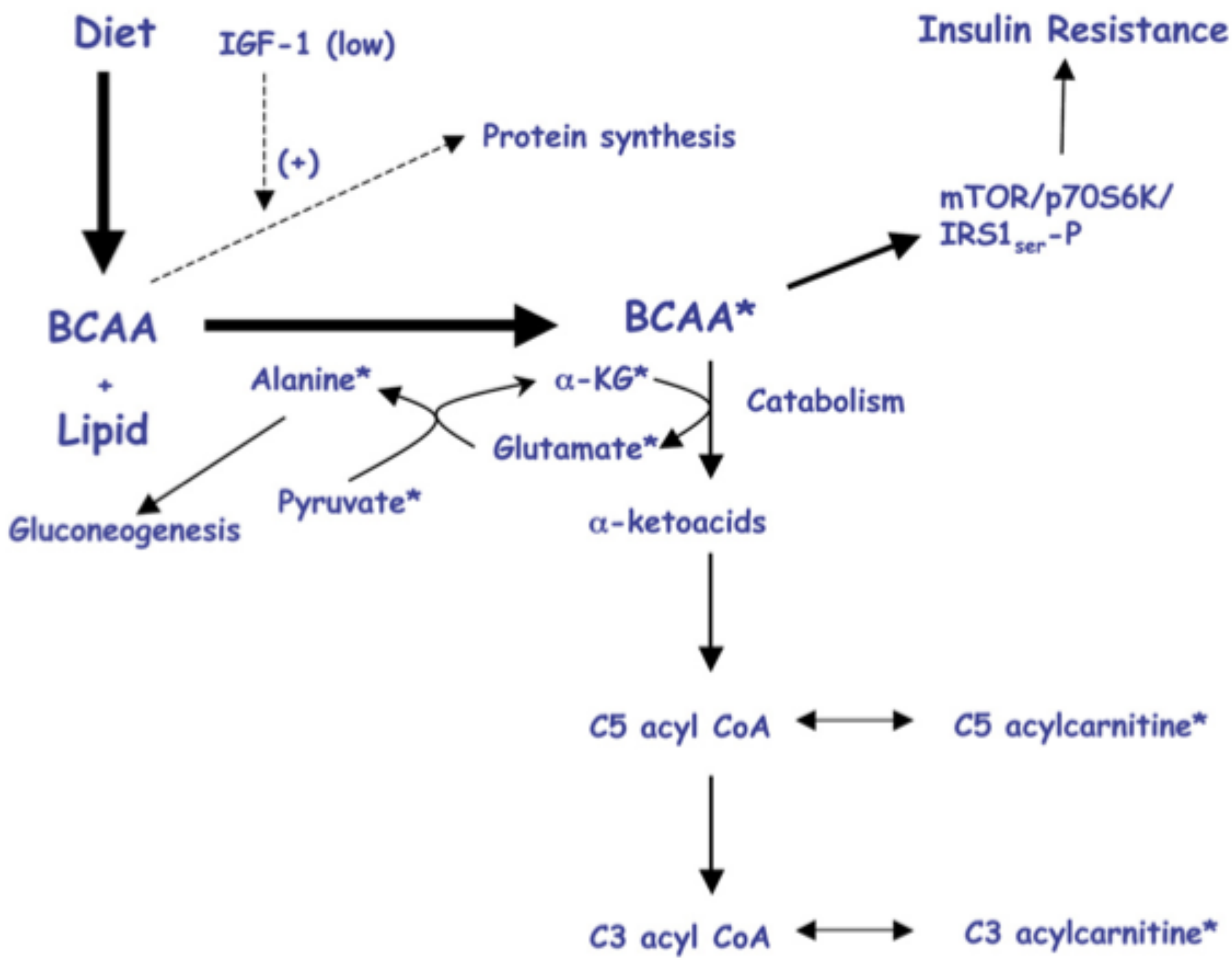


Figure 5. Schematic Summary of BCAA Overload Hypothesis

In the physiological context of overnutrition and low IGF-1 levels, as found in our obese subjects, circulating BCAAs rise, leading to increased flux of these amino acids through their catabolic pathways. We detected changes in several of the intermediary metabolites of the BCAA catabolic pathway in obese subjects, as indicated by the symbol *. A consequence of increased BCAA levels is the activation of the mTOR/S6K1 kinase pathway and phosphorylation of IRS1 on multiple serines, contributing to insulin resistance. In addition, increased BCAA catabolic flux may contribute to increased gluconeogenesis and glucose intolerance via glutamate transamination to alanine.

AAs as predictors of type 2 diabetes

Metabolite profiles and the risk of developing diabetes

Thomas J Wang[1–3], Martin G Larson[3,4], Ramachandran S Vasan[3,5], Susan Cheng[2,3,6], Eugene P Rhee[1,7,8], Elizabeth McCabe[2,3], Gregory D Lewis[1,2,8], Caroline S Fox[3,9,10], Paul F Jacques[11], Céline Fernandez[12], Christopher J O'Donnell[2,3,8], Stephen A Carr[8], Vamsi K Mootha[8,13,14], Jose C Florez[8,13], Amanda Souza[8], Olle Melander[15], Clary B Clish[8] & Robert E Gerszten[1,2,8]

Emerging technologies allow the high-throughput profiling of metabolic status from a blood specimen (metabolomics). We investigated whether metabolite profiles could predict the development of diabetes. Among 2,422 normoglycemic individuals followed for 12 years, 201 developed diabetes. Amino acids, amines and other polar metabolites were profiled in baseline specimens by liquid chromatography–tandem mass spectrometry (LC-MS). Cases and controls were matched for age, body mass index and fasting glucose. Five branched-chain and aromatic amino acids had highly significant associations with future diabetes: isoleucine, leucine, valine, tyrosine and phenylalanine. A combination of three amino acids predicted future diabetes (with a more than fivefold higher risk for individuals in top quartile). The results were replicated in an independent, prospective cohort. These findings underscore the potential key role of amino acid metabolism early in the pathogenesis of diabetes and suggest that amino acid profiles could aid in diabetes risk assessment.

« Five branched-chain and aromatic amino acids had highly significant associations with future diabetes: isoleucine, leucine, valine, tyrosine and phenylalanine. A combination of three amino acids predicted future diabetes (with a more than fivefold higher risk for individuals in top quartile). »

VOLUME 17 | NUMBER 4 | APRIL 2011 NATURE MEDICINE

Visceral obesity is associated with a cluster of metabolic abnormalities

. Hypertriglyceridemia
. Low HDL-cholesterol
. Elevated apolipoprotein B
. Small, dense LDL particles
. Inflammatory profile

. Insulin resistance
. Hyperinsulinemia
. Glucose intolerance
. Impaired fibrinolysis
. Endothelial dysfunction

These features can lead to type 2 diabetes, hypertension and cardiovascular disease

Study Sample

Blood and fat tissue samples obtained from 59 women undergoing gynecological surgery at the CHU de Québec Hospital

Subjects covering the lean to moderately obese range: BMI 20-41 kg/m^2

Non-diabetic, generally low levels of fasting glucose and insulin

Body composition by DEXA, Body fat distribution by CT scan

Full blood lipid profile

Characterization of adipocyte morphology

Biocrates Absolute IDQTM p180 kit was used for metabolite quantification

Boulet et al. 2013, Obesity Society Meeting

Table 1: Anthropometrics, body fat distribution and metabolic characteristics of the women (n=59)

Characteristics	Mean ± SD	Range (min-max)
Age (years)	47.0 ± 4.98	37.7 - 59.4
Weight (kg)	70.6 ± 12.6	53 - 108
BMI (kg/m^2)	27.3 ± 4.7	20.3 - 41.1
Fat mass (kg)	25.8 ± 7.7	12.4 - 47.3
Lean-body mass (kg)	43.8 ± 5.6	35.6 - 58.9
Fat percentage (%)	35.3 ± 5.1	22.7 - 45.1

Blood amino acids as a function of BMI

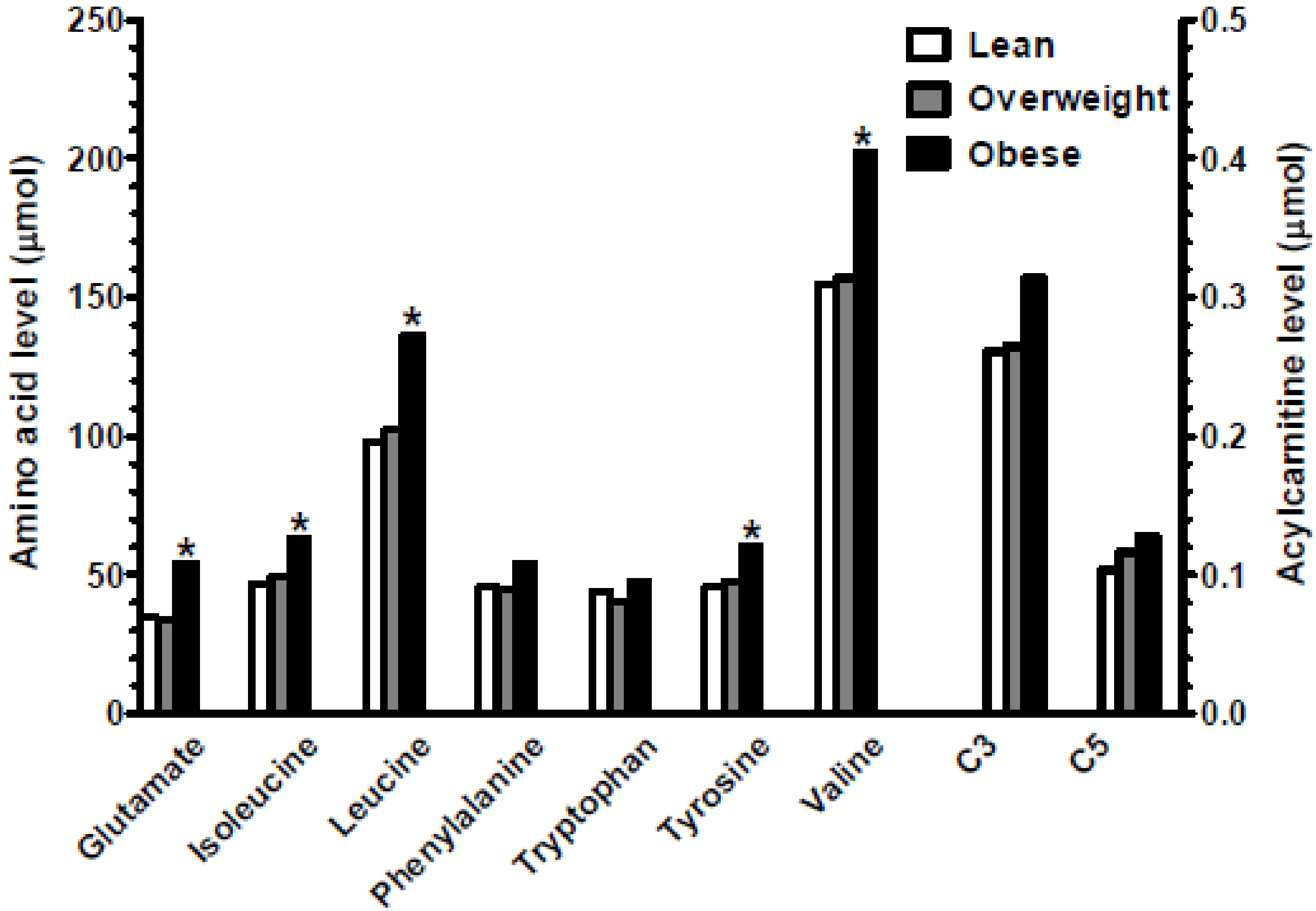
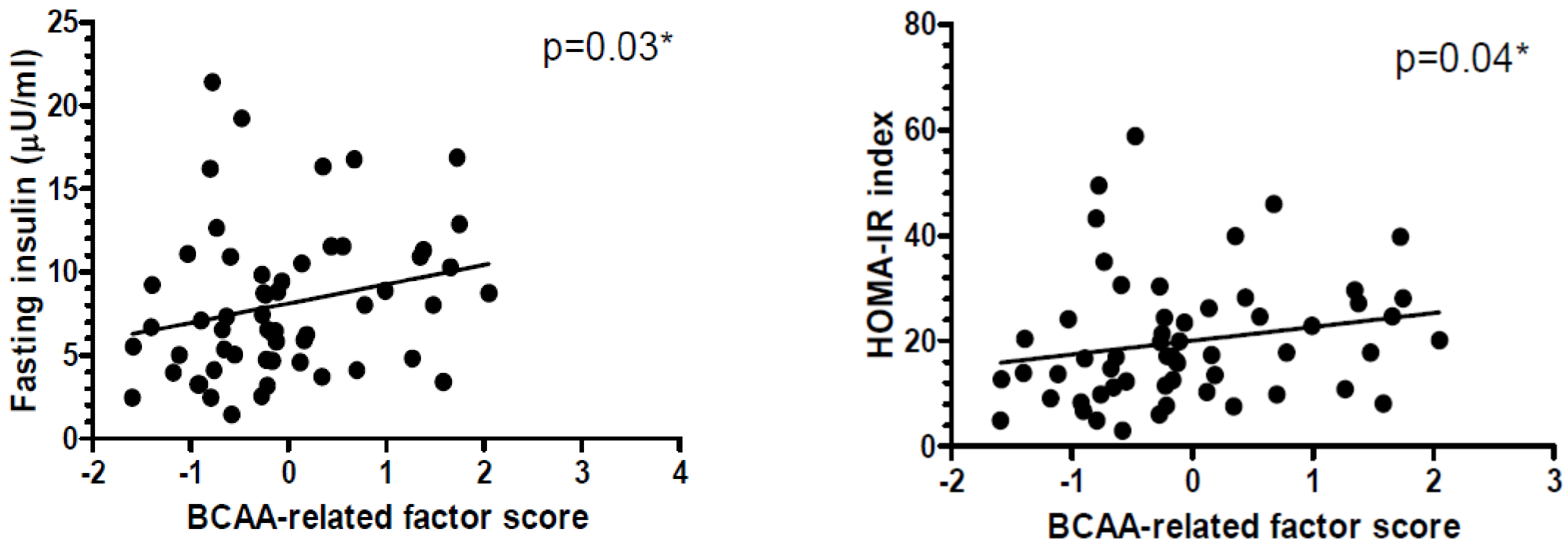


Table 2: Pearson correlations between blood levels of amino acids or acyl carnitines and adiposity markers

	BMI	Fat mass	Visceral AT area	Subcutaneous AT area	Omental adipocyte size	Subcutaneous adipocyte size
Alanine	0.34*	0.31#	0.35*	0.28#	-	0.26#
Arginine	0.27#	0.28#	-	-	-	0.29#
Glutamate	0.28#	-	0.46**§	0.33*	0.26#	-
Isoleucine	0.40*	0.40*	0.28#	0.35*	0.27#	0.32#
Leucine	0.39*	0.37*	0.31#	0.33*	0.30#	0.29#
Lysine	0.26#	0.29#	-	0.27#	-	-
Phenylalanine	0.29#	0.29#	-	-	-	-
Threonine	0.30#	-	-	-	-	-
Tyrosine	0.35*	0.37*	0.31#	0.34*	0.26#	0.31#
Valine	0.34*	0.37*	0.27#	0.31#§	-	-
C3	-	-	0.36*§	-	-	-
C5	0.32*	0.38*	0.31#	0.39*§	-	-

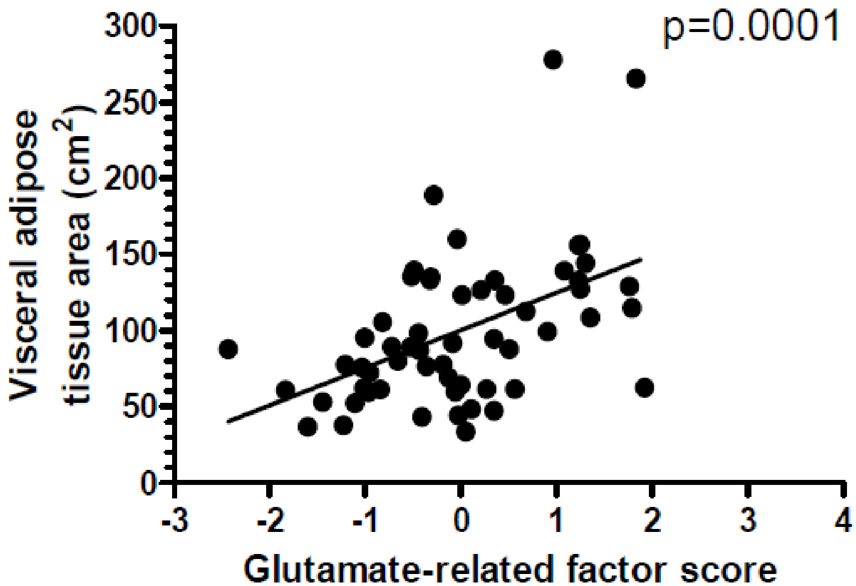
Principal component analysis

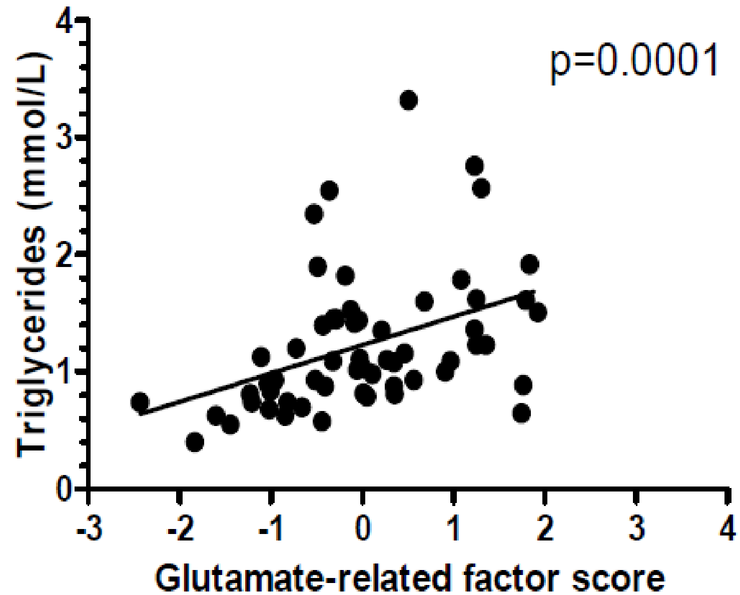
Factor related to blood BCAA levels



Principal component analysis

Factor related to blood glutamate levels





…Observed in patients with low levels of insulin resistance

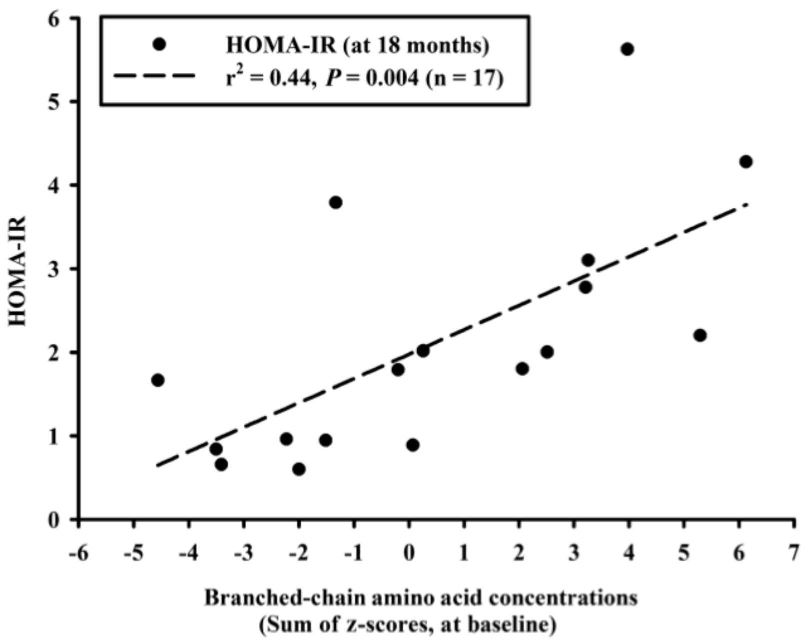
BCAAs as predictors of insulin resistance in children

PEDIATRICOBESITY

ORIGINALRESEARCH doi:10.1111/j.2047-6310.2012.00087.x

Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents

S. E. McCormack[1,2], O. Shaham[3,4], M. A. McCarthy[1], A. A. Deik[3], T. J. Wang[7,8,9], R. E. Gerszten[3,7,8], C. B. Clish[3], V. K. Mootha[3,5,6], S. K. Grinspoon[1] and A. Fleischman[1,2]



What this study adds:

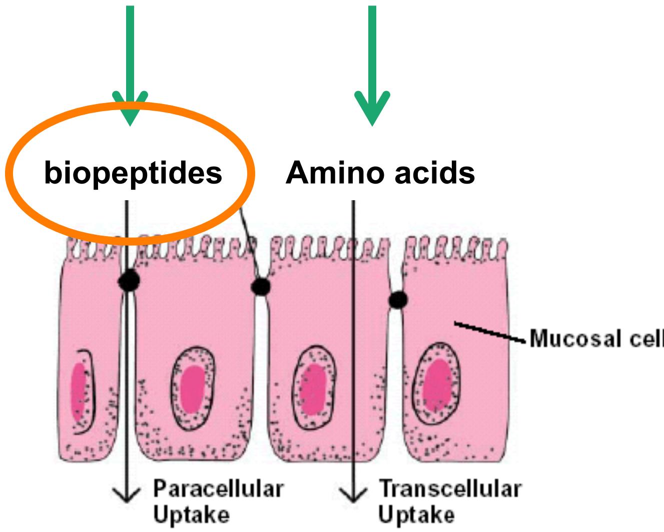
- Increased concentrations of BCAAs are already present in young obese children and their metabolomic profiles are consistent with **increased BCAA catabolism**.
- Elevations in BCAAs in children are positively associated with insulin resistance measured 18 months later, independent of their initial body mass index.

Protéines alimentaires et diabète de type 2

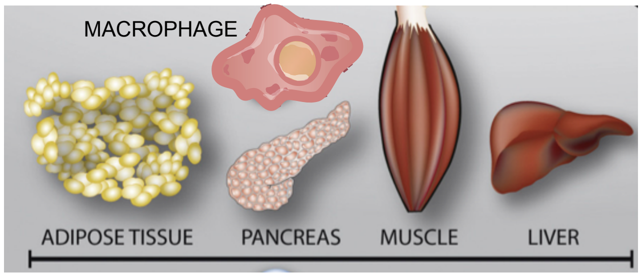
1) Protéines alimentaires et contrôle du diabète de type 2 (DT2)
2) Acides aminés, insulino-résistance et DT2
3) BCAA dans obésité: marqueurs ou causes du DT2 ?
4) **Peptides bioactifs et prévention du DT2**
5) Conclusion et perspectives

Two potential mechanisms underlying the beneficial effects of fish proteins on T2D

Fish proteins



effect on the gut and the microbiota



effect on metabolic tissues and immune cells

Prevention of T2D

Goals of the Project

ω-3 Polyunsaturated Fatty Acids (PUFA)

+

Metabolically Bioactive Peptides (MBPs)

Develop a functional food to improve insulin sensitivity and prevent diabetes

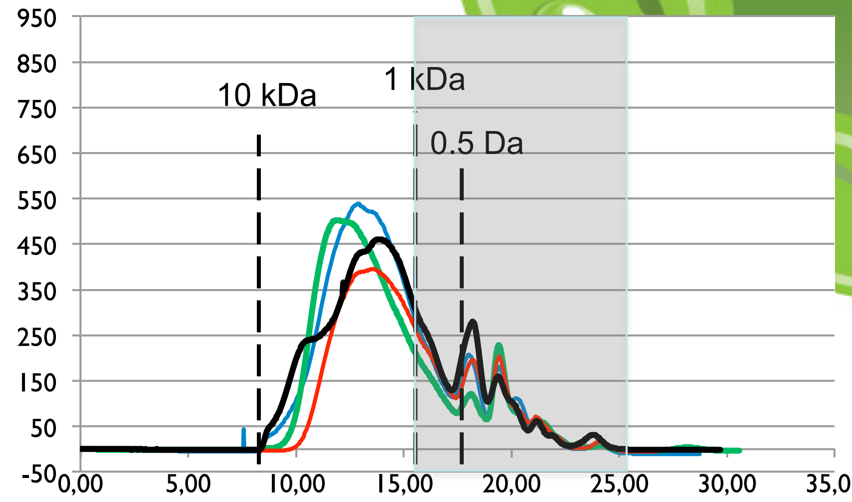
afmnet
Réseau des aliments et des matériaux d'avant-garde
ADVANCED FOODS & MATERIALS NETWORK
Inspiration from the ground up | Aux racines de l'inspiration

Salmon peptide isolation

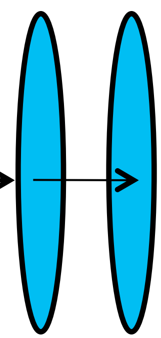
Muscle Protein

- Solubilization
- Enzymatic Digestion

Mixture of Peptides



Ultrafiltration



Salmon Peptides (<1 kDa)

Screening in vitro:

- **insulin-induced glucose uptake in muscle cells**
- **insulin-mediated suppression of hepaotocyte glucose production**
- **inhibition of macrophage inflammation**

LDLr $^{-/-}$ mice

When fed a high fat (55% kcal) and sucrose diet (HFHS) containing cholesterol (0.2%), these mice are prone to develop obesity and dyslipidemia, leading to T2D and CVD

HFHS 10% SPH Fish oil 10% SPH + Fish oil

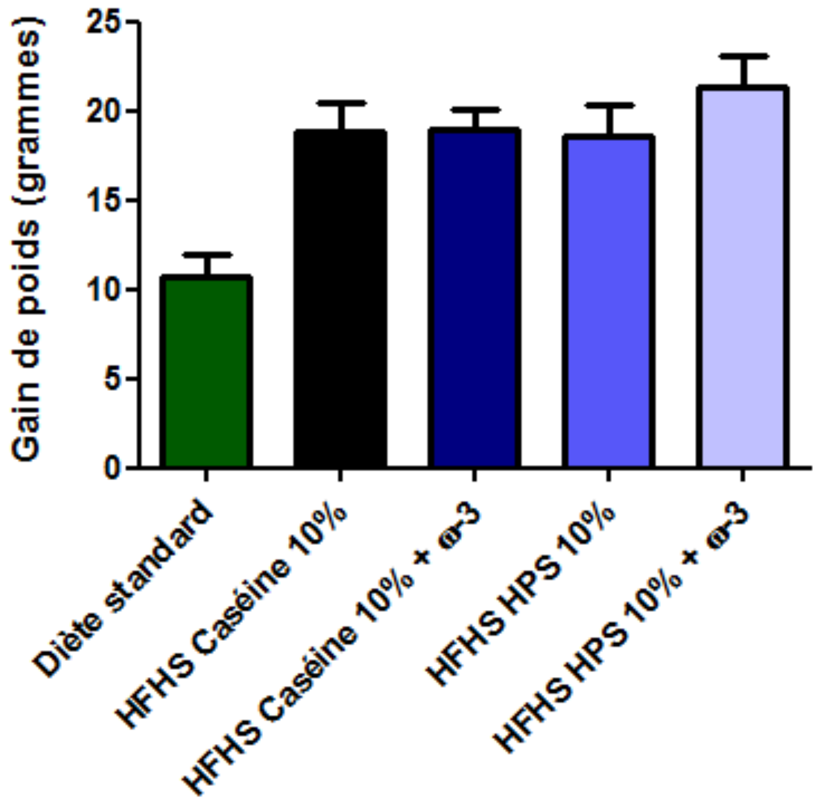
12-24 weeks

Daily gavage

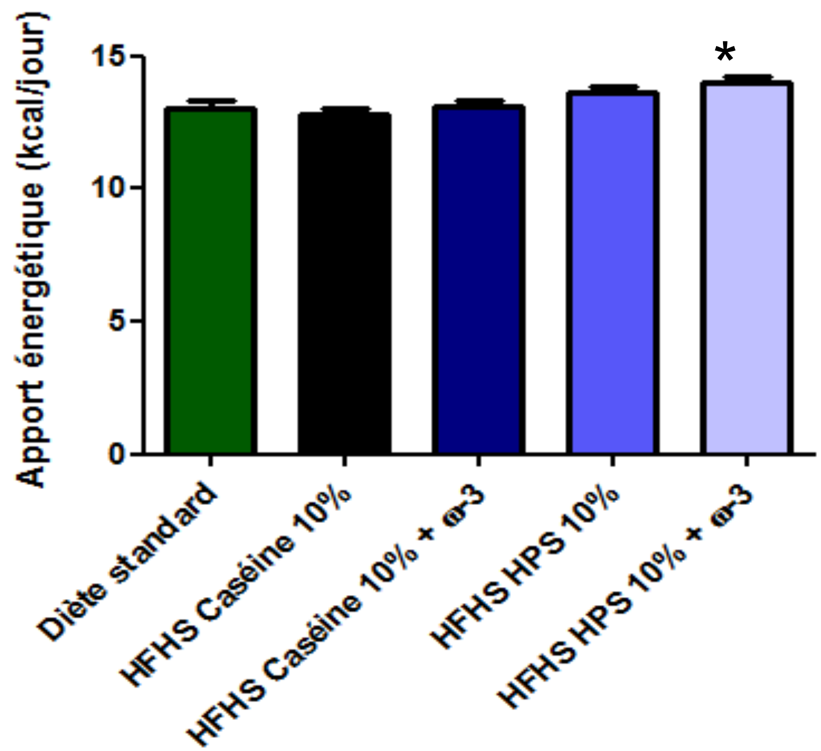
Impact on obesity-linked glucose intolerance and dyslipidemia

Body weight gain and energy intake of LDLr$^{-/-}$/ApoB^{100} mice after 12 weeks on HFHS diet supplemented with or without salmon peptides and/or fish oil

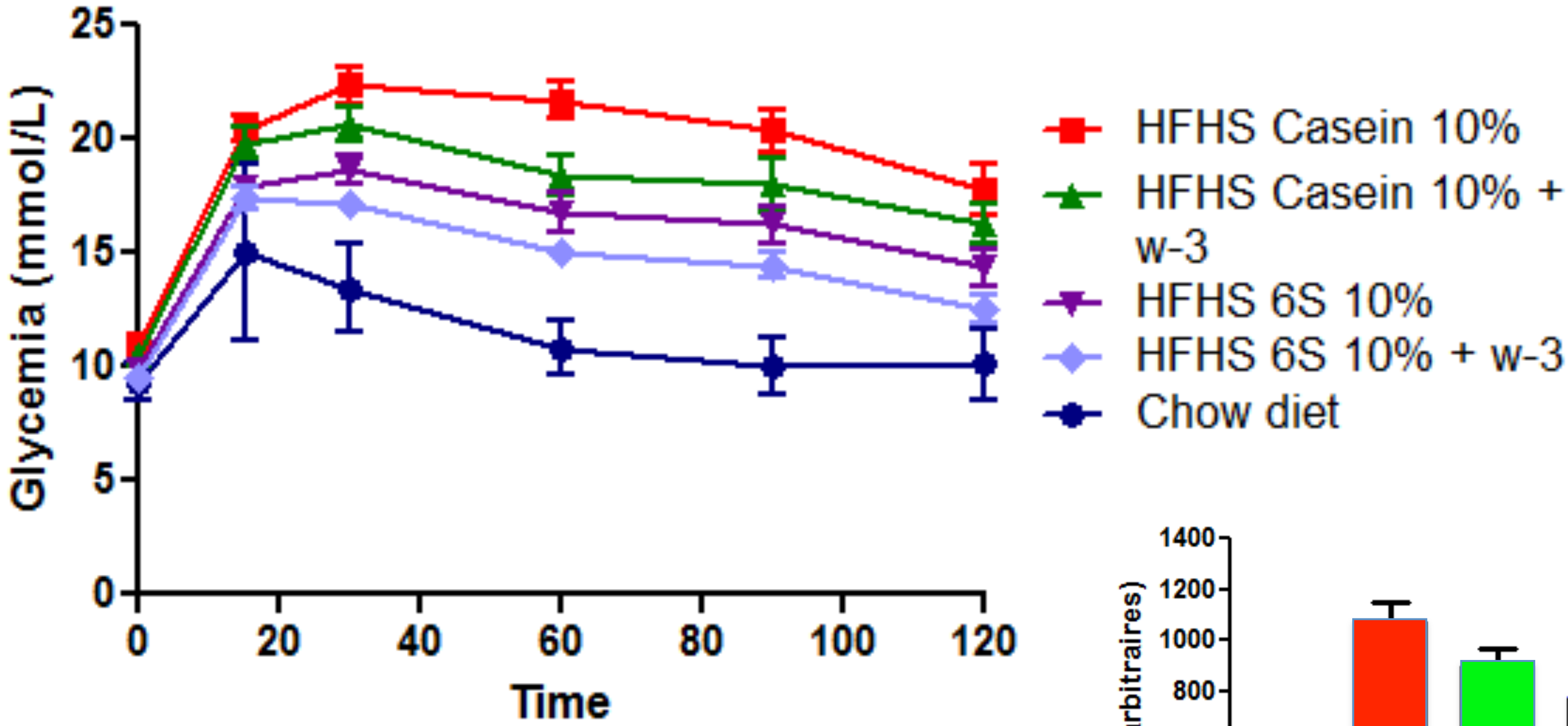
Body weight gain(g)

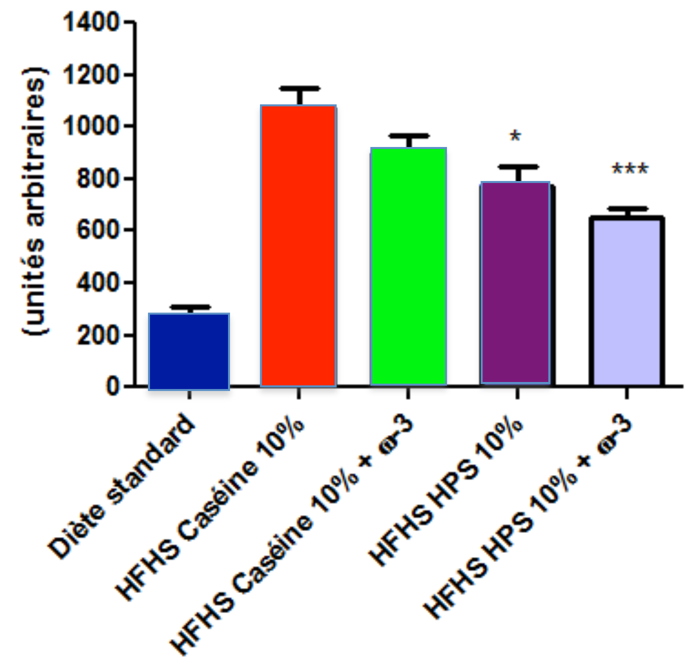


Energy intake (kcal/jd)

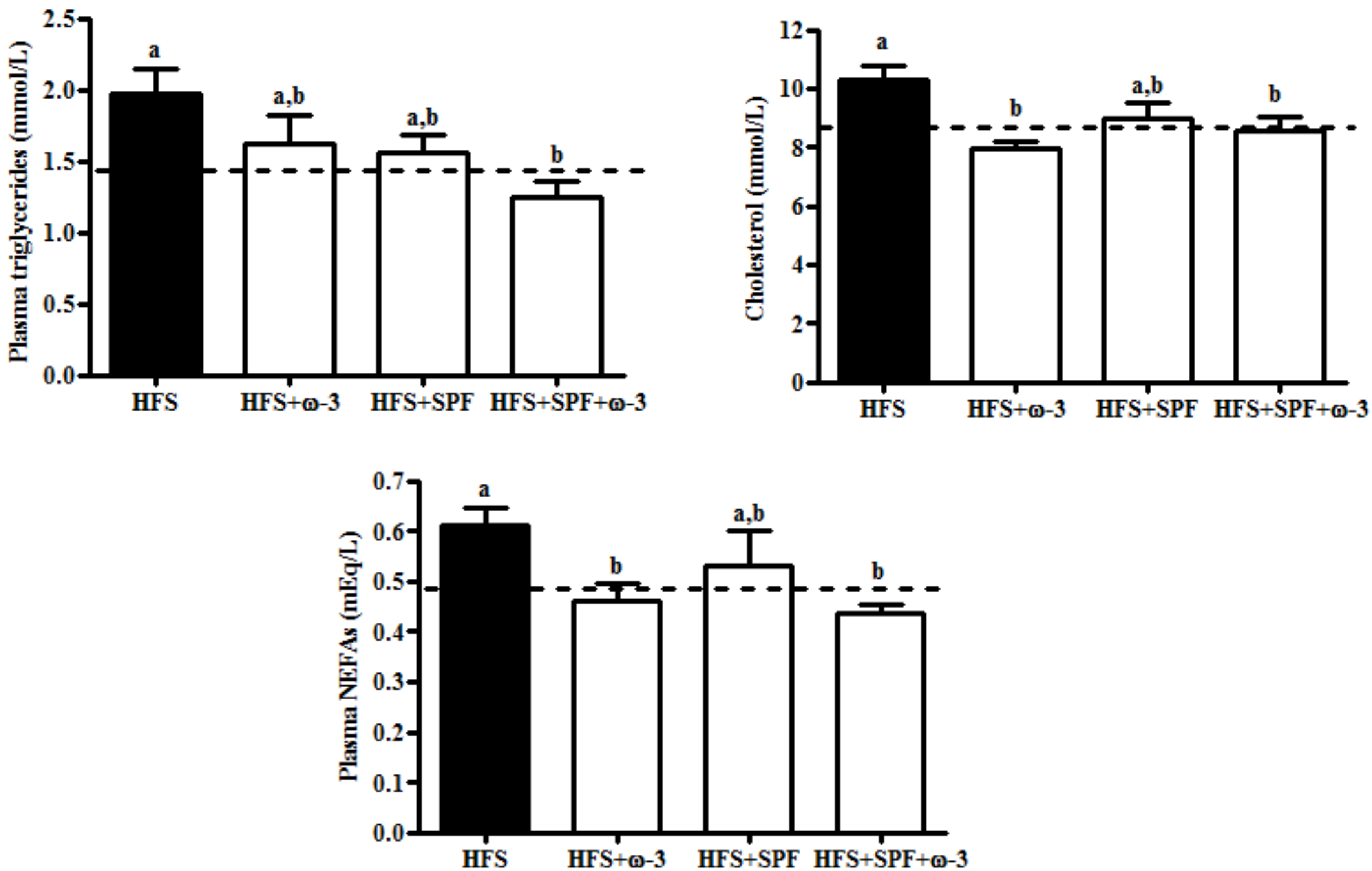


The addition of salmon peptides and/or fish oil improve glucose tolerance in HFHS fed LDLr$^{-/-}$/ApoB^{100} mice

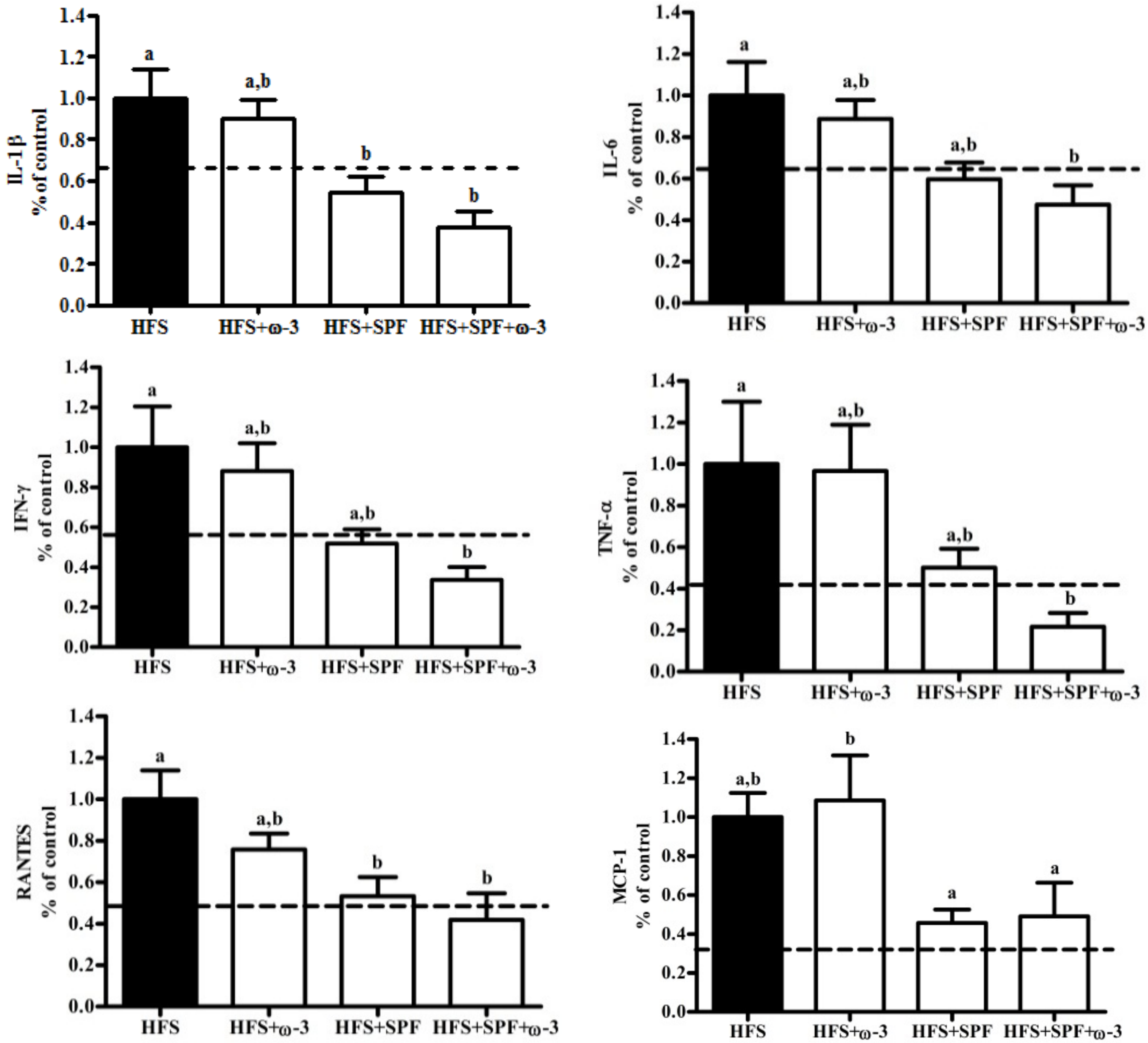




Dyslipidemia in obese LDLr KO mice is reduced by fish oil and the salmon protein fraction



Adipose tissue inflammation is reduced by fish oil and the salmon protein fraction



Current Goals of the research program

2011-2015

1. Identify Novel Bioactive salmon ***Peptides***
2. Pre-clinical Validation in Animal Models
3. Clinical trial with functional food enriched with BFPs
4. Commercialization of functional foods containing n-3 PUFA and BFPs

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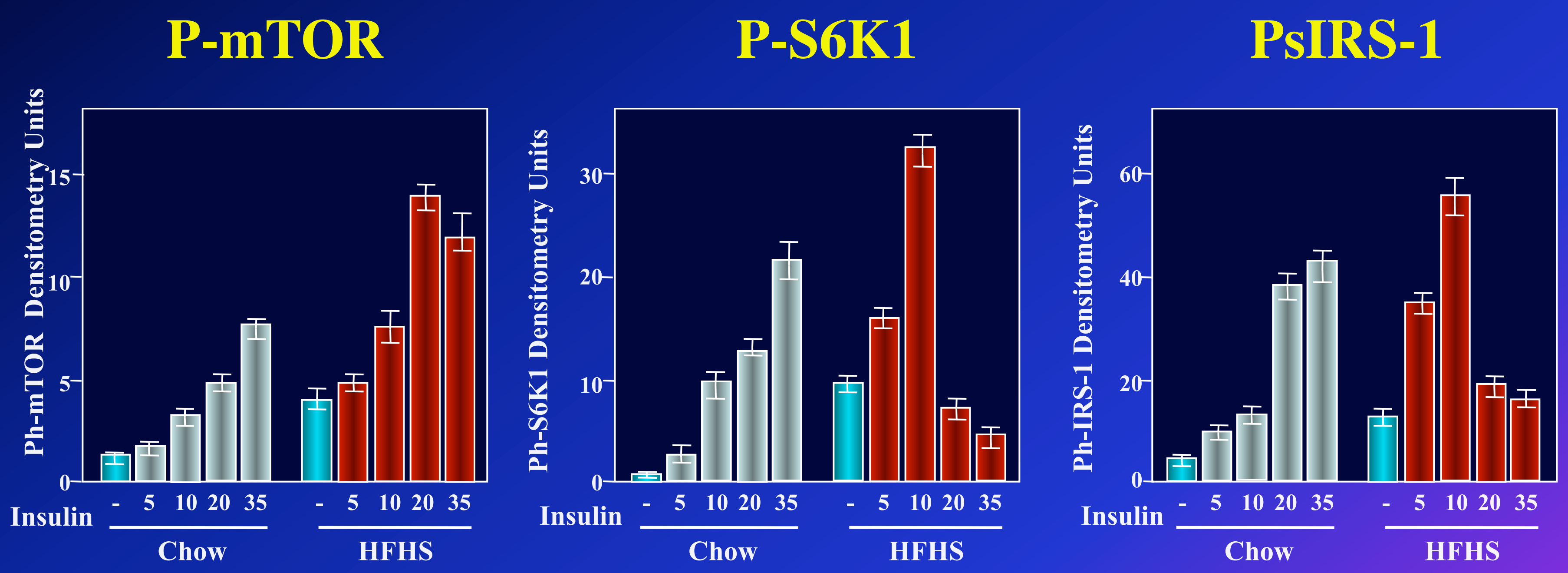
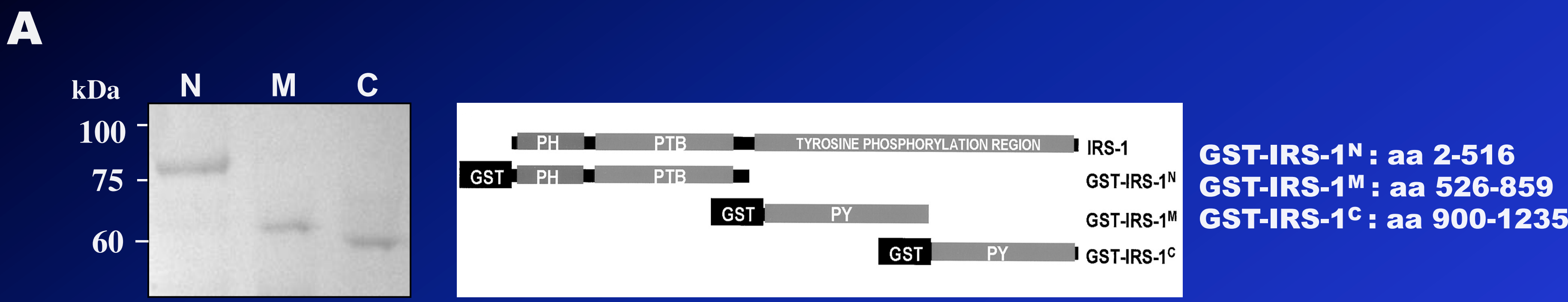
Canadian Diabetes Association
Leading the fight against diabetes

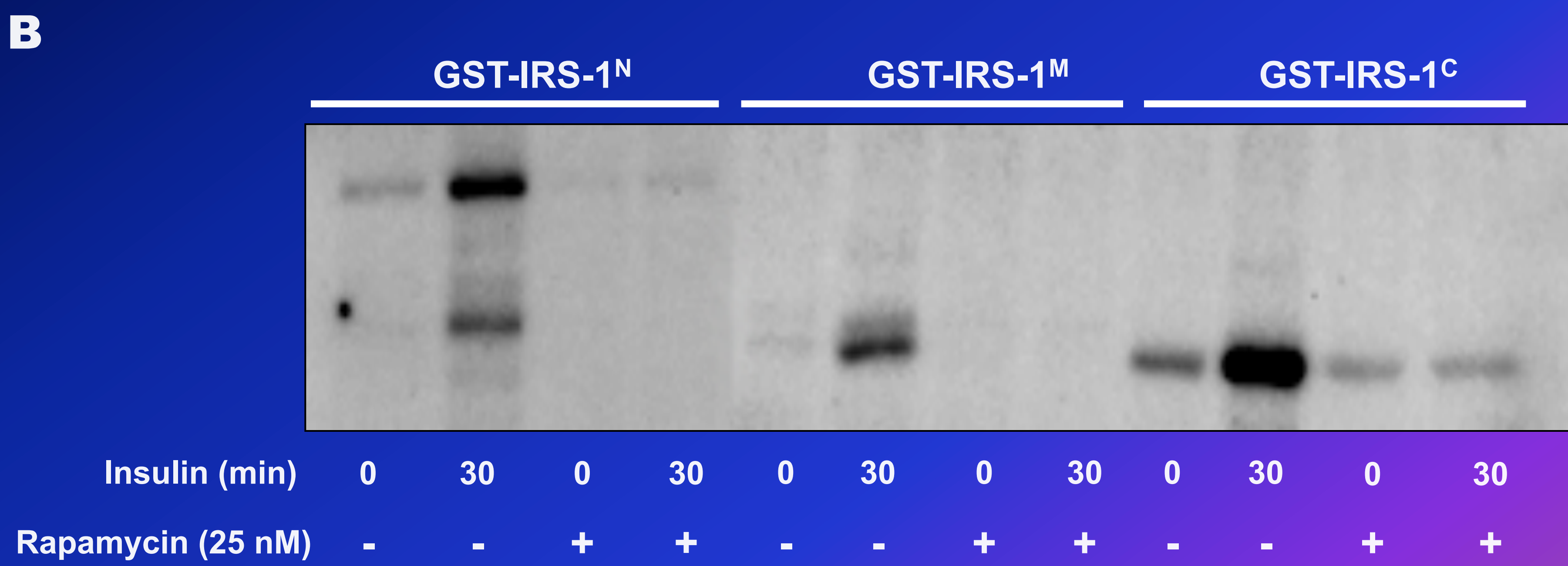
CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

mTOR/S6K1 activation and IRS-1 serine phosphorylation in liver of high-fat fed obese rats



S6K1 phosphorylates GST-IRS-1 in vitro





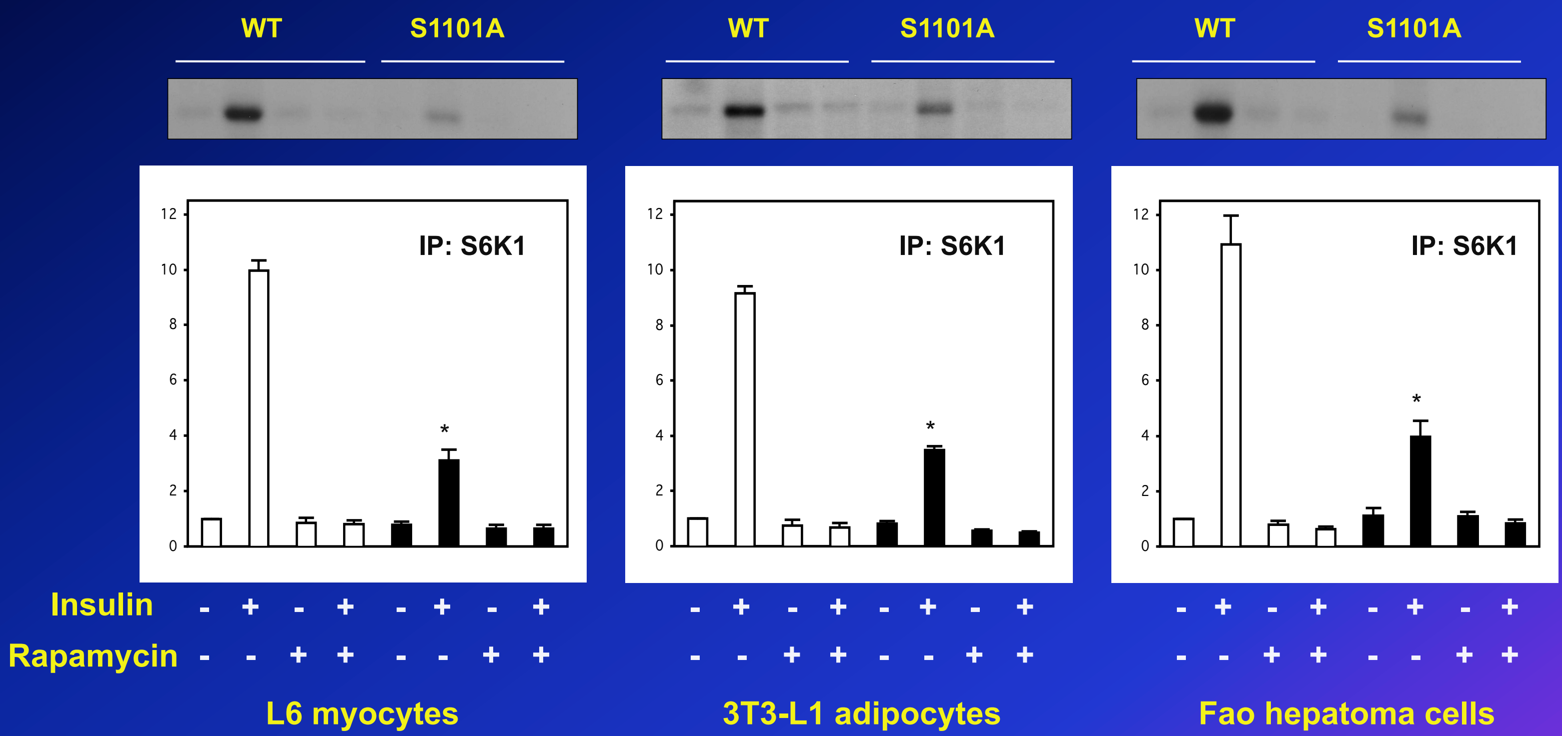
Potential sites in IRS-1 phosphorylated by S6K1 based on motif recognition.

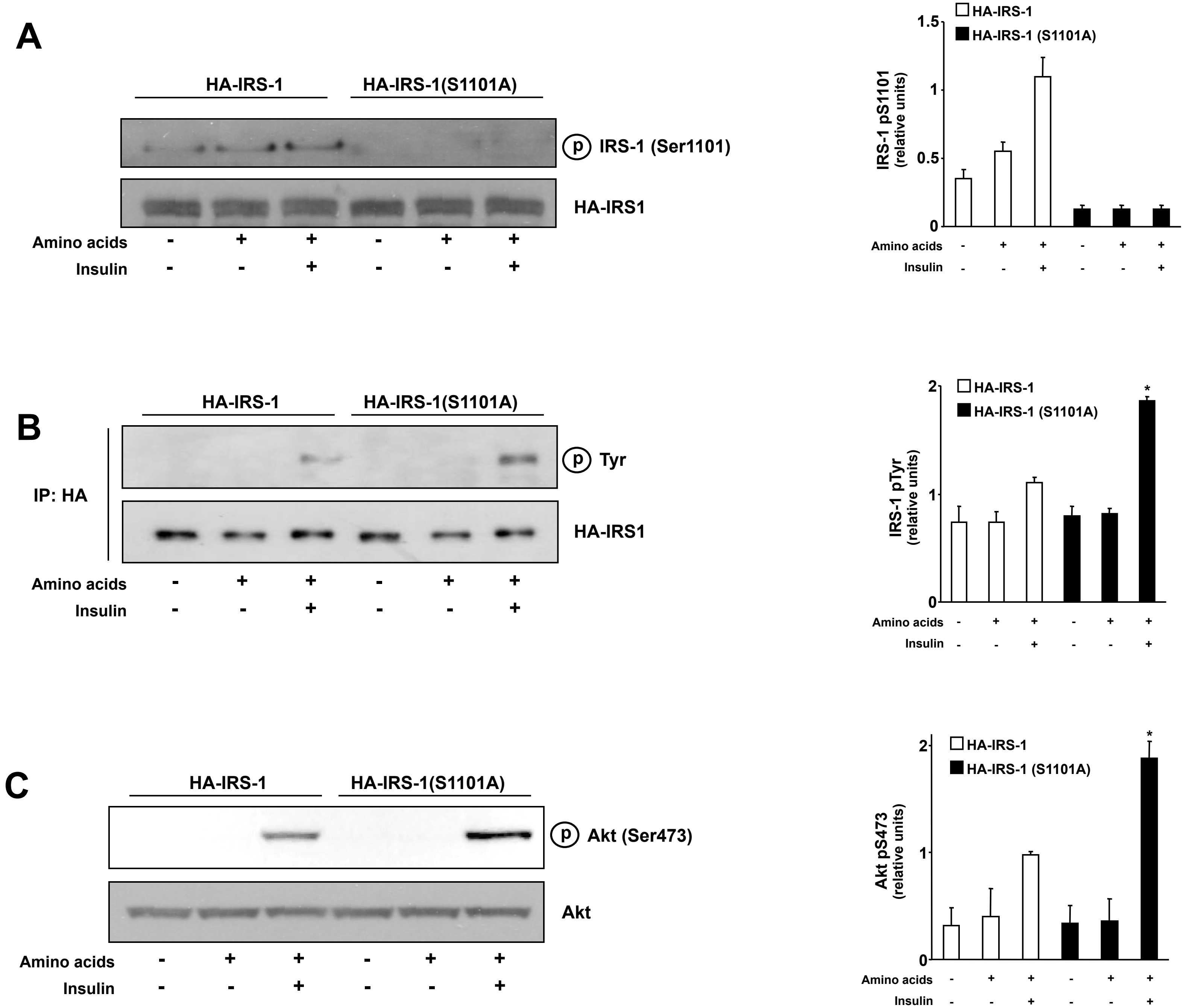
S6K1 recognition motif (K/RxRxxS) in GST-IRS-1

Residues (rat IRS-1)

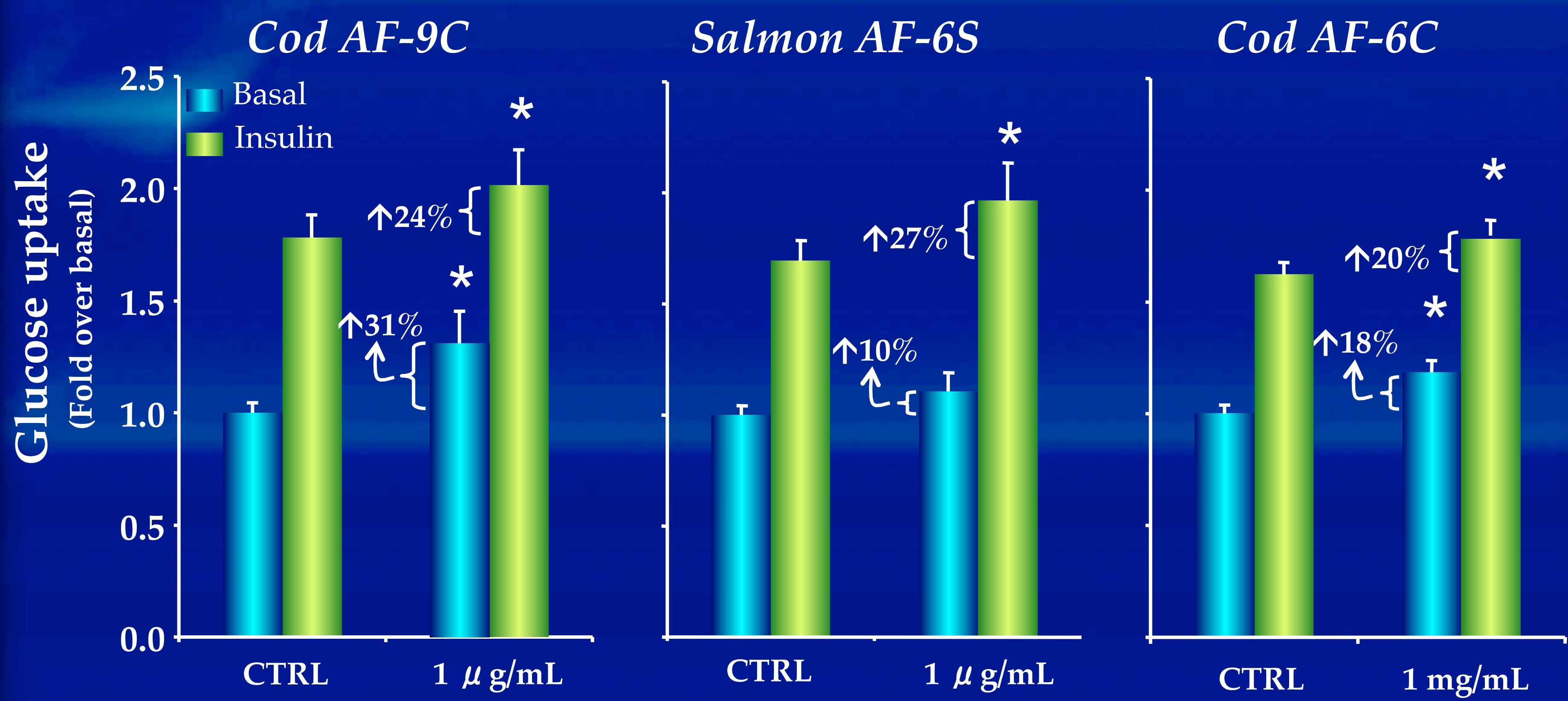
Residues	Construct
Ser57 Ser265 Ser302 Ser325 Ser358	GST-IRS-1^{N}
Ser1101	GST-IRS-1^{C}

identification of Ser1101 as an S6K1-mediated phosphorylation site in IRS-1



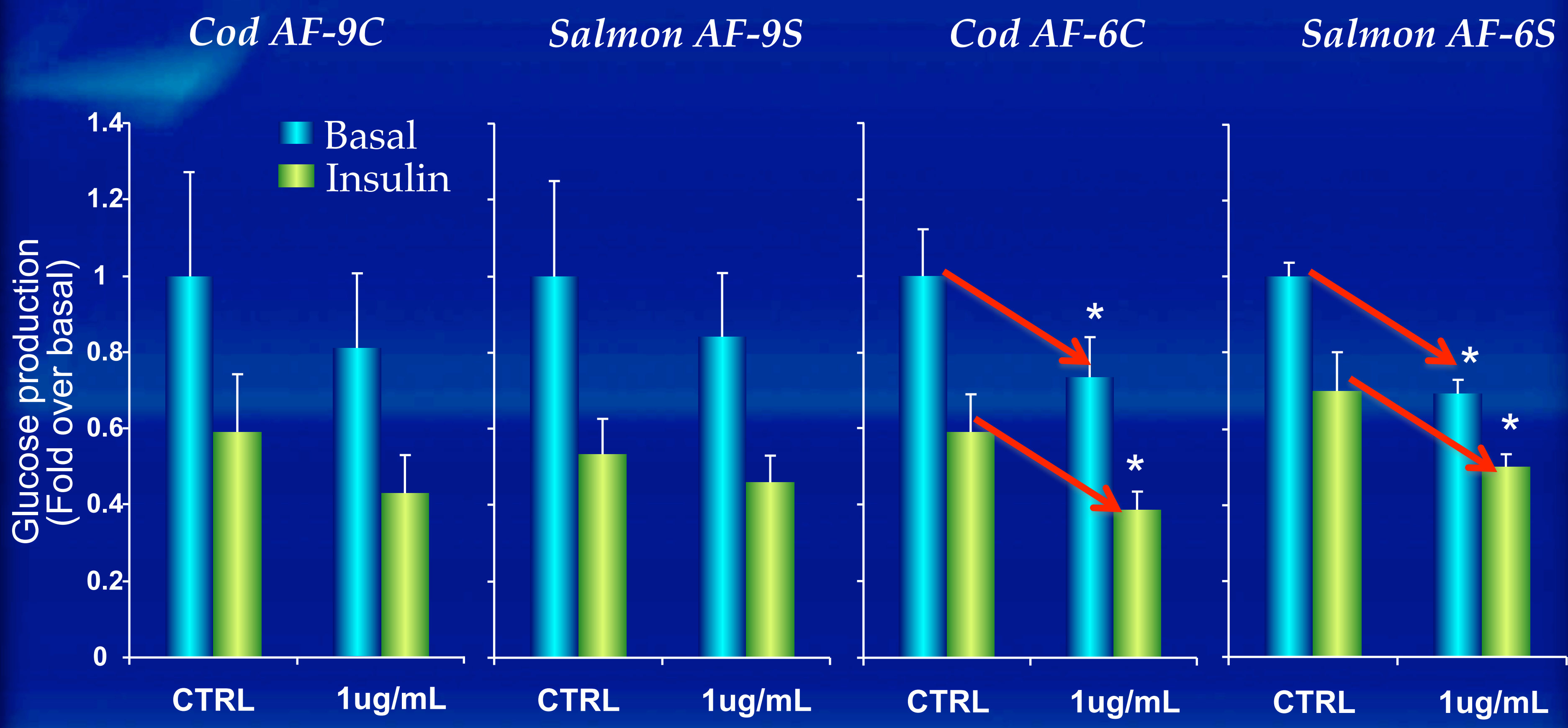

A
HA-IRS-1
HA-IRS-1(S1101A)
p IRS-1 (Ser1101)
HA-IRS1
Amino acids - + + - + +
Insulin - - + - - +
HA-IRS-1
HA-IRS-1 (S1101A)
IRS-1 pS1101 (relative units)
1.5
1
0.5
0
Amino acids - + + - + +
Insulin - - + - - +
B
HA-IRS-1
HA-IRS-1(S1101A)
IP: HA
p Tyr
HA-IRS1
Amino acids - + + - + +
Insulin - - + - - +
HA-IRS-1
HA-IRS-1 (S1101A)
IRS-1 pTyr (relative units)
2
1
0
*
Amino acids - + + - + +
Insulin - - + - - +
C
HA-IRS-1
HA-IRS-1(S1101A)
p Akt (Ser473)
Akt
Amino acids - + + - + +
Insulin - - + - - +
HA-IRS-1
HA-IRS-1 (S1101A)
Akt pS473 (relative units)
2
1
0
*
Amino acids - + + - + +
Insulin - - + - - +

Effect of fish peptides on glucose uptake in L6 myocytes



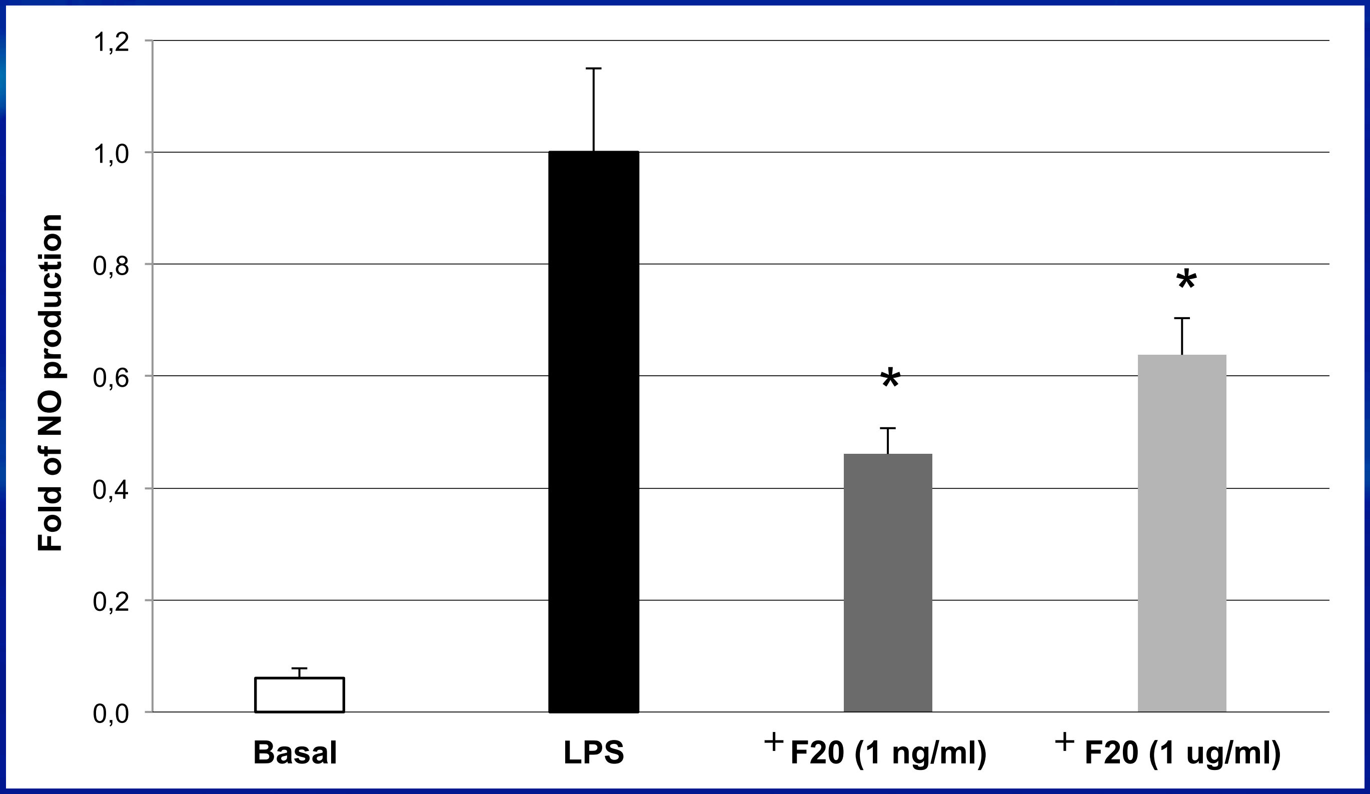
The values represent the means ± SEM of 5-9 experiments.

Effect of fish peptides on hepatic glucose production in cultured hepatocytes



The values represent the means ± SEM of 4 experiments.

Salmon F20 fraction (AF-6S) reduces iNOS activity in macrophages



Cell PRESS

Cell Metabolism
Perspective

Interplay between Lipids and Branched-Chain Amino Acids in Development of Insulin Resistance

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