

Seminario all'Università di Pavia

Pavia, 4 Novembre 2013

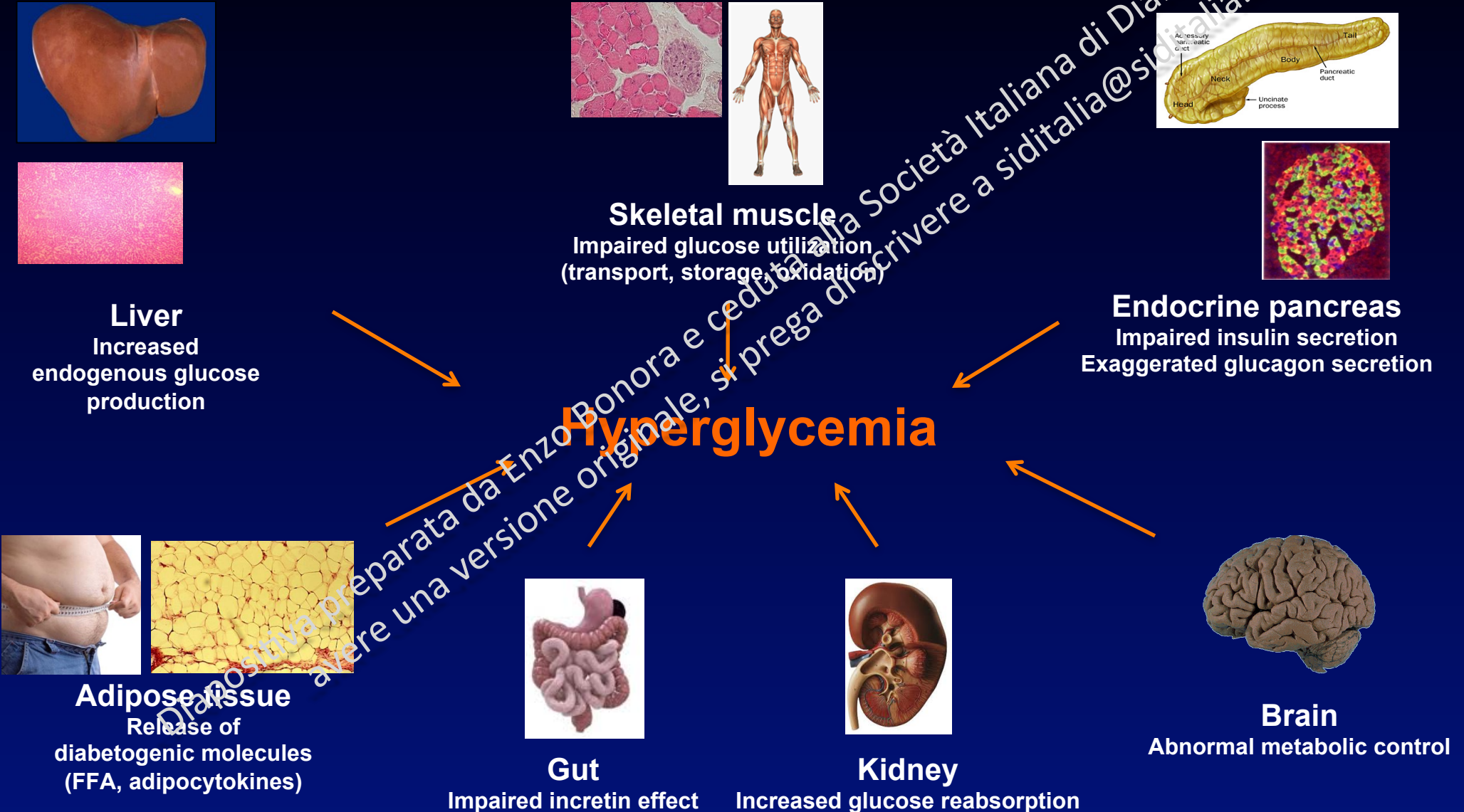
Insulino-resistenza e deficit beta-cellulare: chi è più importante nella patogenesi del diabete tipo 2?

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T2DM: a Complex and Systemic Pathogenesis



Key Pathogenetic Defects of T2DM



Diapostitmo preparata da Enzo Bonora e ceduta alla Società Italiana di Diabetologia per
una versione originale, si prega di scrivere a siditalia@siditalia.it

➤ **Quesito:** Chi è più importante fra insulino-resistenza e deficit beta-cellulare per lo sviluppo di diabete tipo 2?

➤ **Risposta:** Il deficit beta-cellulare ma....

➤ **Evidenze:** studi genetici, studi epidemiologici, studi di fisiopatologia clinica, studi su modelli animali e umani

Diapositiva preparata da Enzo Bonora e ceduta alla Società Italiana di Diabetologia. Per avere una versione originale, si prega di scrivere a siditalia@siditalia.it

Studi genetici

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Genes Environment

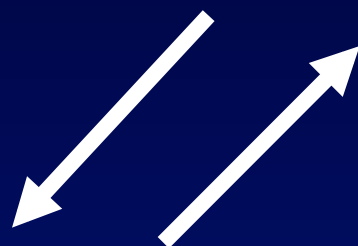


**Beta-cell
Dysfunction**

Genes Environment



**Insulin
Resistance**



Diabetes Milieu:

Hyperglycemia, Dyslipidemia, Inflammation, Oxidant stress

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Diabete tipo 2: malattia poligenica

Geni della disfunzione beta cellulare

- KCNJ11
- TCF7L2
- IGF2BP2
- WFS1
- SLC30A8
- CDC123
- TSPAN8
- KCNQ1

- MTNR1B
- HHEX
- CDKAL1
- CDKN2A/B
- HNF1B
- JAZF1
- GCKR

Geni dell'insulinoresistenza

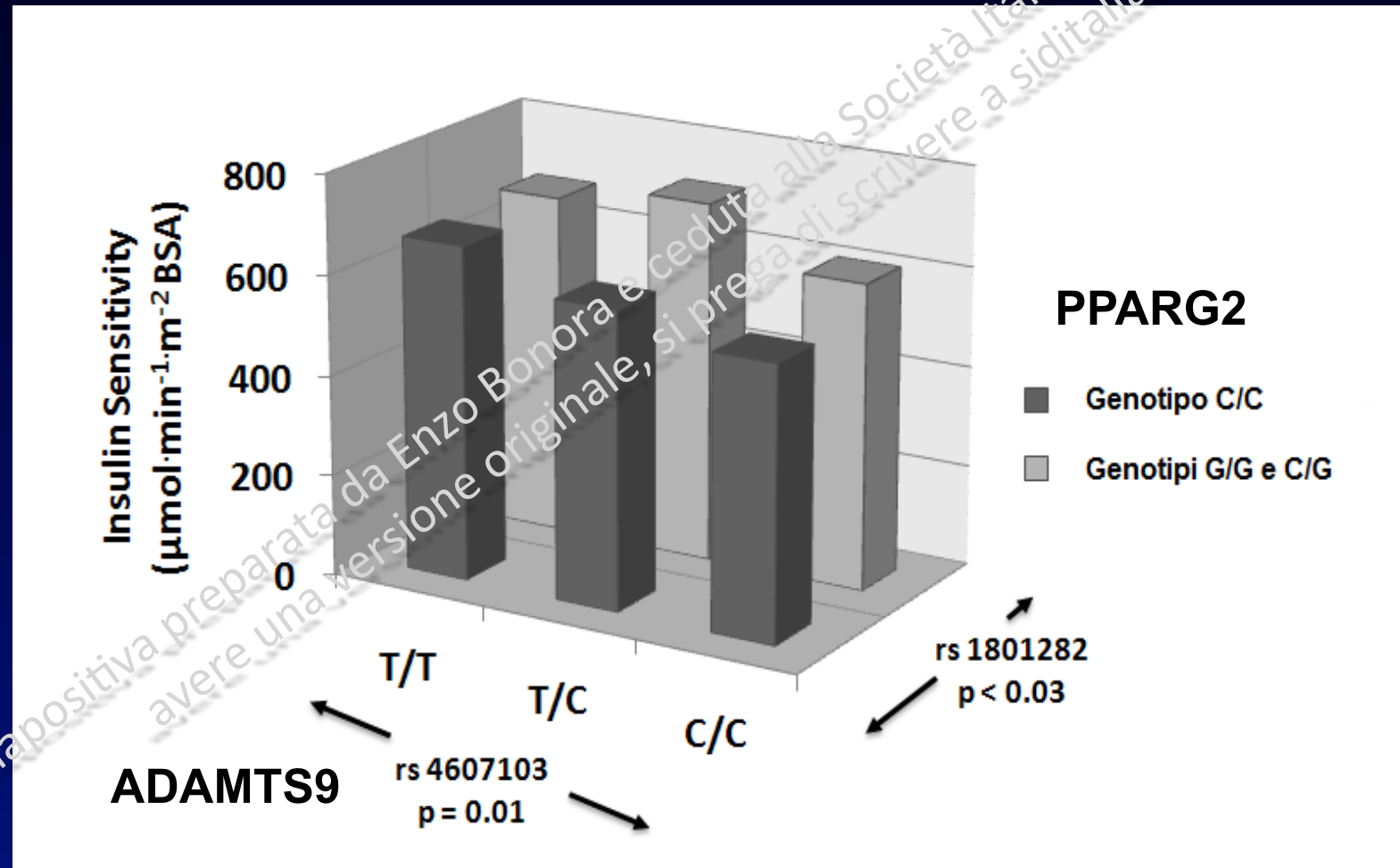
- ENPP1 (PC-1)
- PPARG
- ADAMTS9

Diapositiva preparata da Enzo Bonora e ceduta alla Società Italiana di Diabetologia per avere una versione originale, si prega di scrivere a siditalia@siditalia.it



Sensibilità insulinica (M-clamp) nei soggetti diabetici del VNDS in funzione della presenza o meno dell'allele di rischio di PPARG2 e dell'allele di rischio di ADAMTS9

VNDS – Trombetta et al - Acta Diabetologica 50: 401-2013



Association of T2DM with Common Genetic Variants

Ahlqvist E – Clin Chem 2011; 57: 241-254

Table 1. Genetic variants associated with T2D.

Varlants	Nearest gene	Full gene name	OR T2D	Study type ^a	References
rs4607103, rs6795735	ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9	1.09–1.1	MA	Voight et al. (41), Zeggini et al. (53)
rs1552224	ARAP1 (CENTD2)	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1	1.13–1.14	MA	Voight et al. (41)
rs243021	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	1.08–1.09	MA	Voight et al. (41)
rs2975760, rs3792267	CAPN10	Calpain 10	1.17	LA	Weedon et al. (20)
rs12779790	CDC123/CAMK1D	Cell division cycle 123 homolog-calcium/calmodulin-dependent protein kinase ID	1.09	MA	Voight et al. (41), Zeggini et al. (53)
rs7754840, rs10440833	CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	1.12–1.25	GWAS	Saxena et al. (32), Scott et al. (33), Voight et al. (41), Steinthorsdottir et al. (44)
rs13292136	CHCHD9/TLE4	Coiled-coil-helix-coil-coiled-helix domain containing 9/transducin-like enhancer of split 4	1.11–1.20	MA	Voight et al. (41)
rs10811661, rs10965250	CDKN2A/2B	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)–cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1.20	GWAS	Saxena et al. (32), Scott et al. (33), Voight et al. (41)
rs5945326	DUSP9	Dual specificity phosphatase 9	1.27	MA	Voight et al. (41)
rs1111875, rs5015480	HHEX	Hematopoietically expressed homeobox	1.13–1.18	GWAS	Saxena et al. (32), Voight et al. (41), Sladek et al. (43)
rs1531343	HMG2	High mobility group AT-hook 2	1.10–1.20	MA	Voight et al. (41)
rs7957197	HNF1A	HNF1 homeobox A	1.07–1.14	MA	Voight et al. (41)
rs1920792, rs7501939, rs757210, rs4430796	HNF1B	HNF1 homeobox B	1.1–1.17	CS	Windler et al. (15), Voight et al. (41), Gudmundsson et al. (22)
rs2334499	HCCA2	YY1 associated protein 1	1.35	GWAS	Kong et al. (52)
rs4402960, rs1470579	IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2	1.14	GWAS	Saxena et al. (32), Scott et al. (33), Voight et al. (41)
rs2943641, rs7578326	IRS1	Insulin receptor substrate 1	1.09–1.12	CS	Almind et al. (25), Voight et al. (41)
rs864745, rs849134	JAZF1	JAZF zinc finger 1	1.12–1.13	MA	Voight et al. (41), Zeggini et al. (53)
rs5219	KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11	1.13–1.14	CS	Hani et al. (28), Saxena et al. (32), Voight et al. (41)
rs2237892, rs163184, rs231362	KCNQ1	Potassium voltage-gated channel, Kv1-like subfamily, member 1	1.08–1.23	GWAS	Voight et al. (41), Unoki et al. (48), Yasuda et al. (49)
rs972283	KLF14	Krüppel-like factor 14	1.07–1.1	MA	Voight et al. (41)
rs10923931	NOTCH2/ADAMTS1	Notch 2–ADAM metalloproteinase domain 30	1.14	MA	Voight et al. (41), Zeggini et al. (53)
rs1801282, rs13081389	PPARG	Peroxisome proliferator-activated receptor gamma	1.14–1.24	CS	Deeb et al. (21), Saxena et al. (32), Voight et al. (41)

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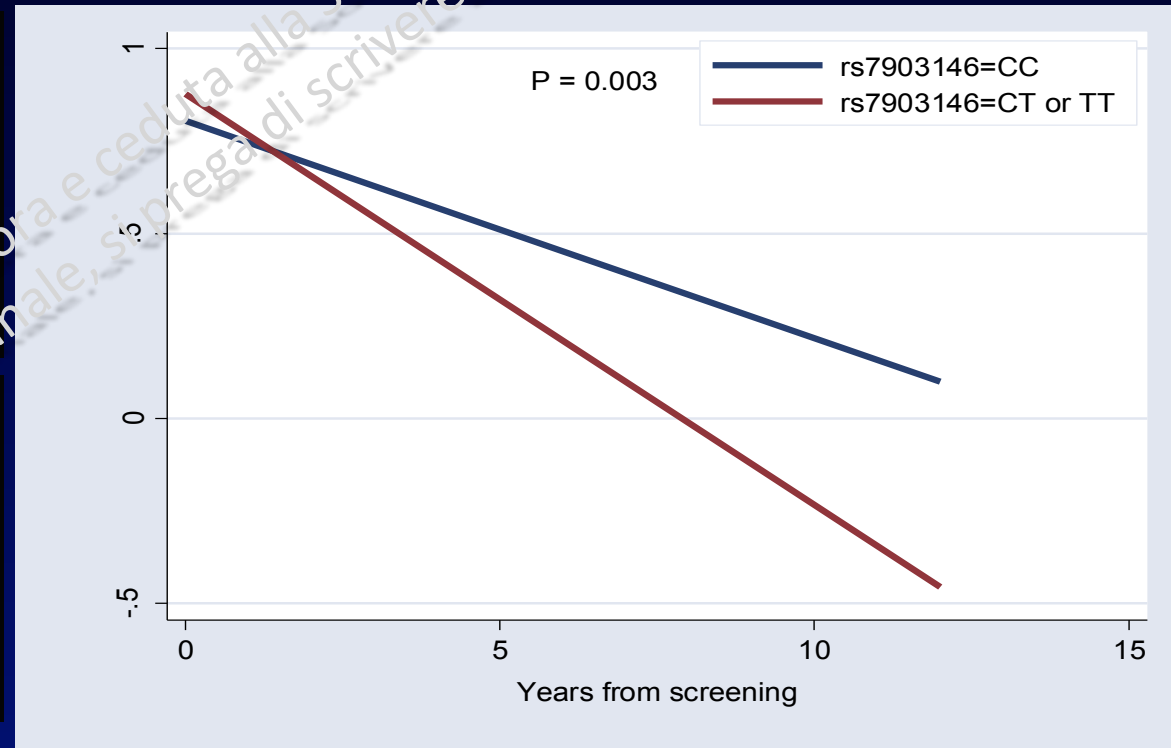
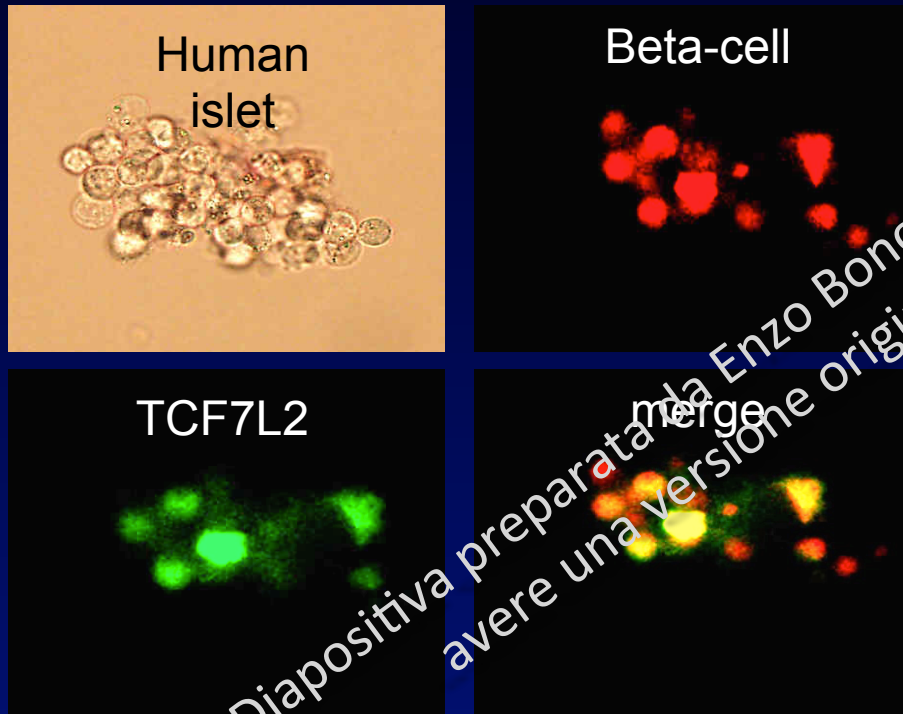
Table 1. Genetic variants associated with T2D. (Continued from page 243)

Varlants	Nearest gene	Full gene name	OR T2D	Study type ^a	Reference
rs8042680	PRC1	Protein regulator of cytokinesis 1	1.07–1.1	MA	Voight et al. (41)
rs17584499	PTPRD	Protein tyrosine phosphatase, receptor type, D	1.57	GWAS	Tsai et al. (51)
rs7593730	RBMS1	RNA binding motif, single stranded interacting protein 1	1.11	MA	Qi et al. (54)
rs13266634, rs11558471, rs3802177	SLC30A8	Solute carrier family 30 (zinc transporter), member 8	1.12–1.15	GWAS	Saxena et al. (32), Voight et al. (41), Sladek et al. (43), Dupuis et al. (55)
rs391300	SRR	Serine racemase	1.28	GWAS	Tsai et al. (51)
rs7903146, rs12255372, rs4506565, rs11196218	TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	1.4	LA	Grant et al. (12), Saxena et al. (32), Voight et al. (41)
rs7578597, rs11899863	THADA	Thyroid adenoma associated	1.15–1.17	MA	Voight et al. (41), Zeggini et al. (53)
rs896854	TP53INP1	Tumor protein p53 inducible nuclear protein 1	1.06–1.1	MA	Voight et al. (41)
rs7961581, rs4760790	TSPAN8-LGR5	Tetraspanin 8–leucine-rich repeat-containing G protein-coupled receptor 5	1.11	MA	Voight et al. (41), Zeggini et al. (53)
rs10010131, rs6446482, rs1801214	WFS1	Wolfram syndrome 1 (wolframin)	1.11–1.13	CS	Minton et al. (35), Sandhu et al. (36), Voight et al. (41)
rs4457053	ZBED3	Zinc finger, BED-type containing 3	1.08–1.16	MA	Voight et al. (41)
rs11634397	ZFAND6	Zinc finger, AN1-type domain 6	1.06–1.11	MA	Voight et al. (41)

^a MA, metaanalysis; LA, linkage analysis; CS, candidate study.

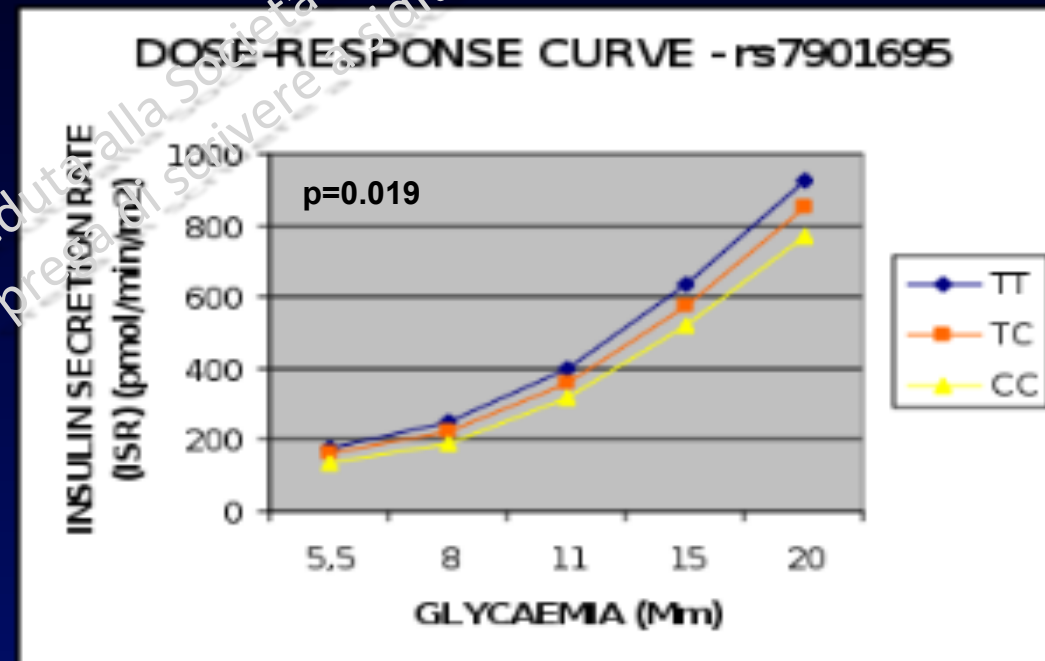
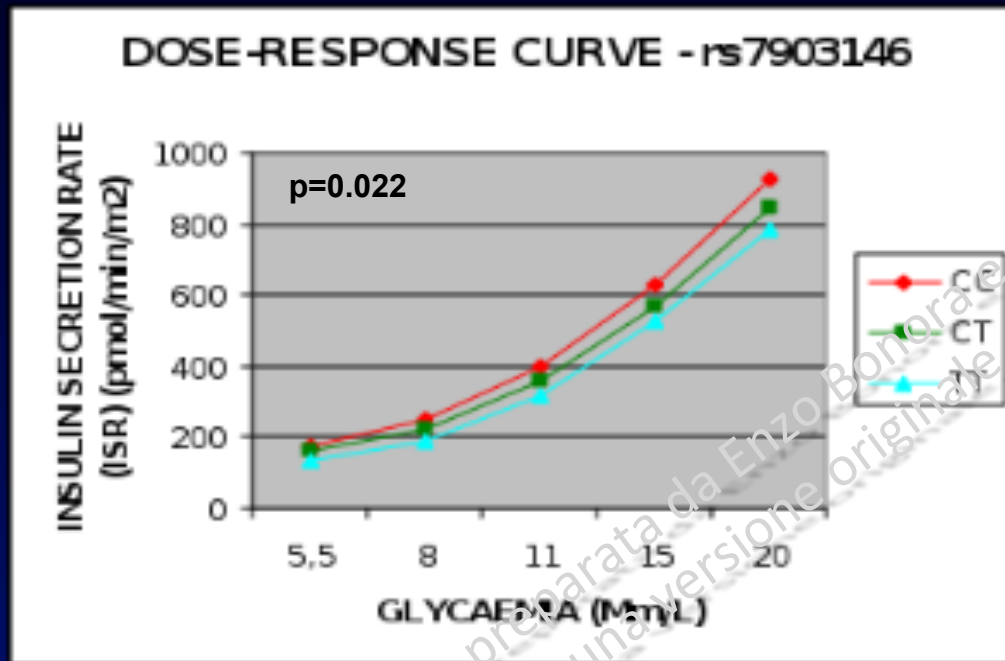
TCF7L2 is expressed in human islets and risk genotypes are associated to deterioration of β -cell function over time

Lyssenko et al – J Clin Invest 2007; 117: 2155-2163



Association of rs7901695 and rs7903146 Risk Alleles of TCF7L2 with Glucose Stimulated Insulin Secretion in Newly-Diagnosed T2DM

VNDS - Bonetti et al – JCE&M 2011; 96: E389

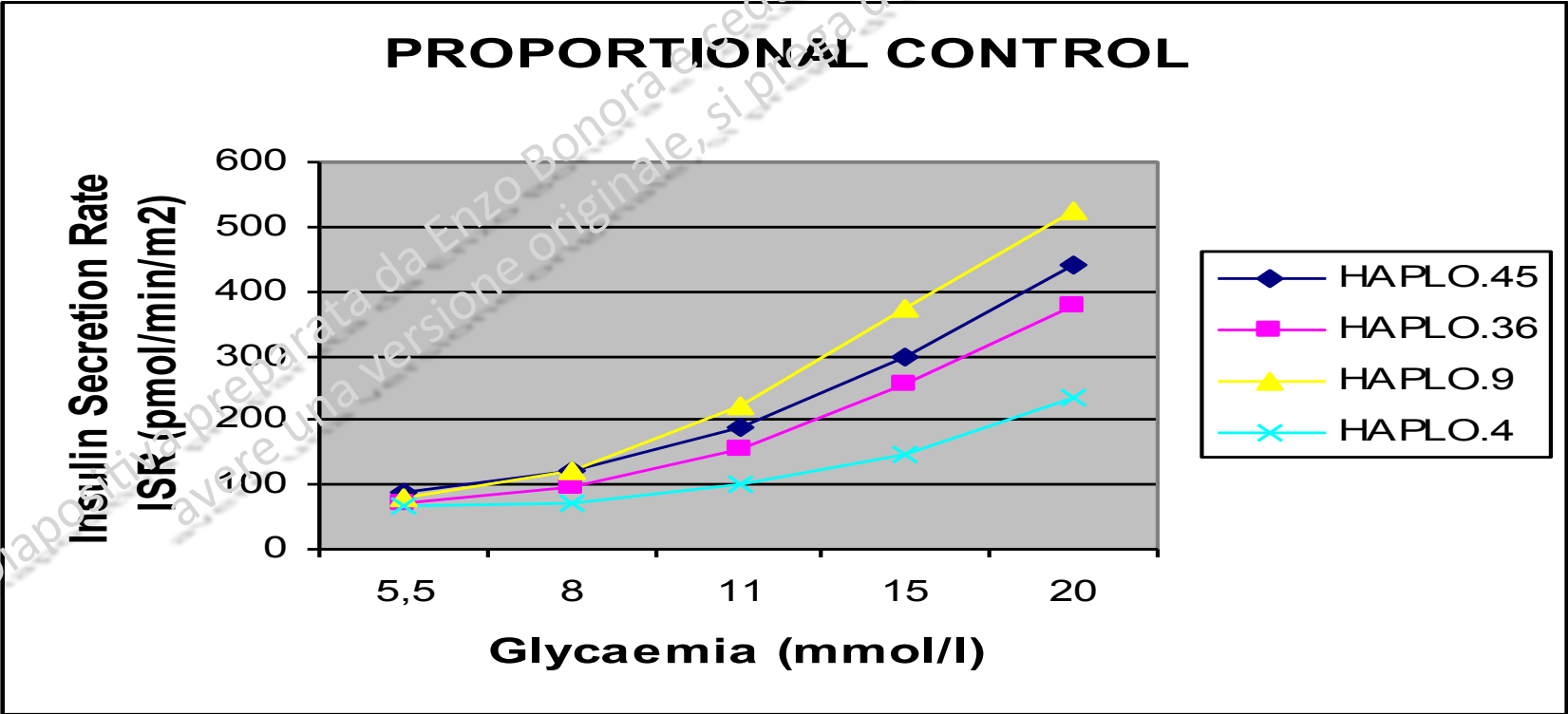


N=464

Haplotype analysis of TCF7L2 polymorphisms and glucose stimulated insulin secretion in newly-diagnosed T2DM

VNDS - Bonetti et al – JCE&M 2011; 96: E389

	rs7901695	rs7903146	rs11196205	rs12255372	Haplotype frequency
haplo.4	C	T	C	G	0,0383
haplo.36	C	T	C	T	0,3479
haplo.9	T	C	C	G	0,0864
haplo.45	T	C	C	G	0,4559

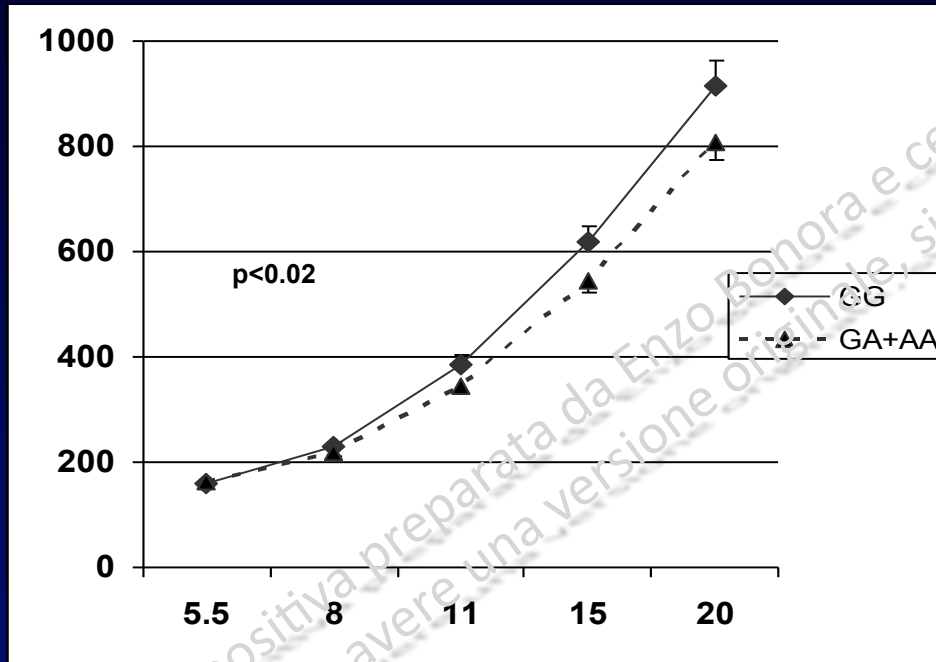


Association of rs6717980 and rs2384628 risk alleles of *GCKR* with glucose stimulated insulin secretion in newly diagnosed T2DM

VNDS - Bonetti et al – Diabetes Care 2011

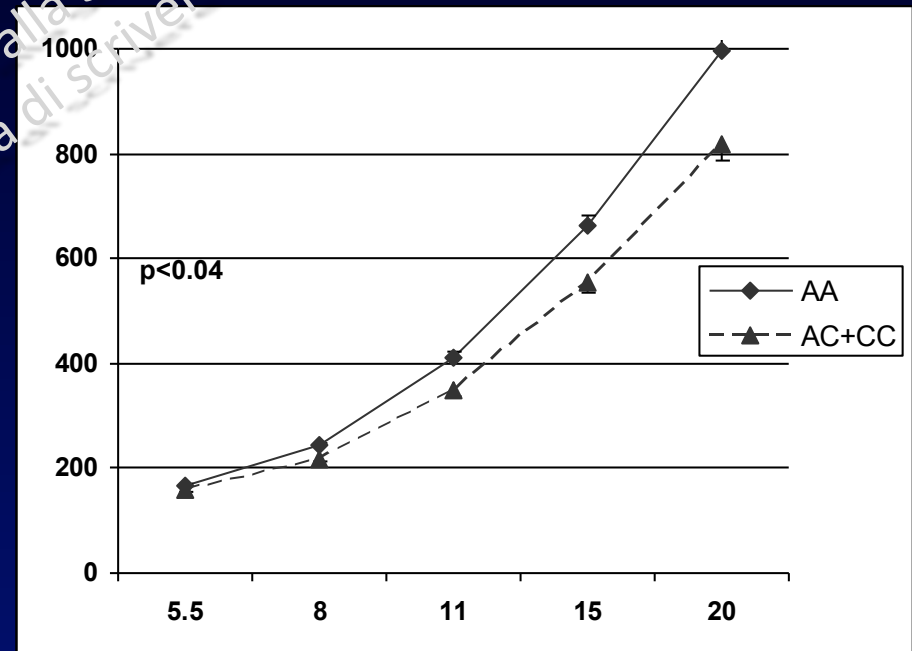
rs6717980

INSULIN SECRETION RATE (ISR)
[pmol·min⁻¹·m⁻² BSA]



Plasma glucose (mmol/l)

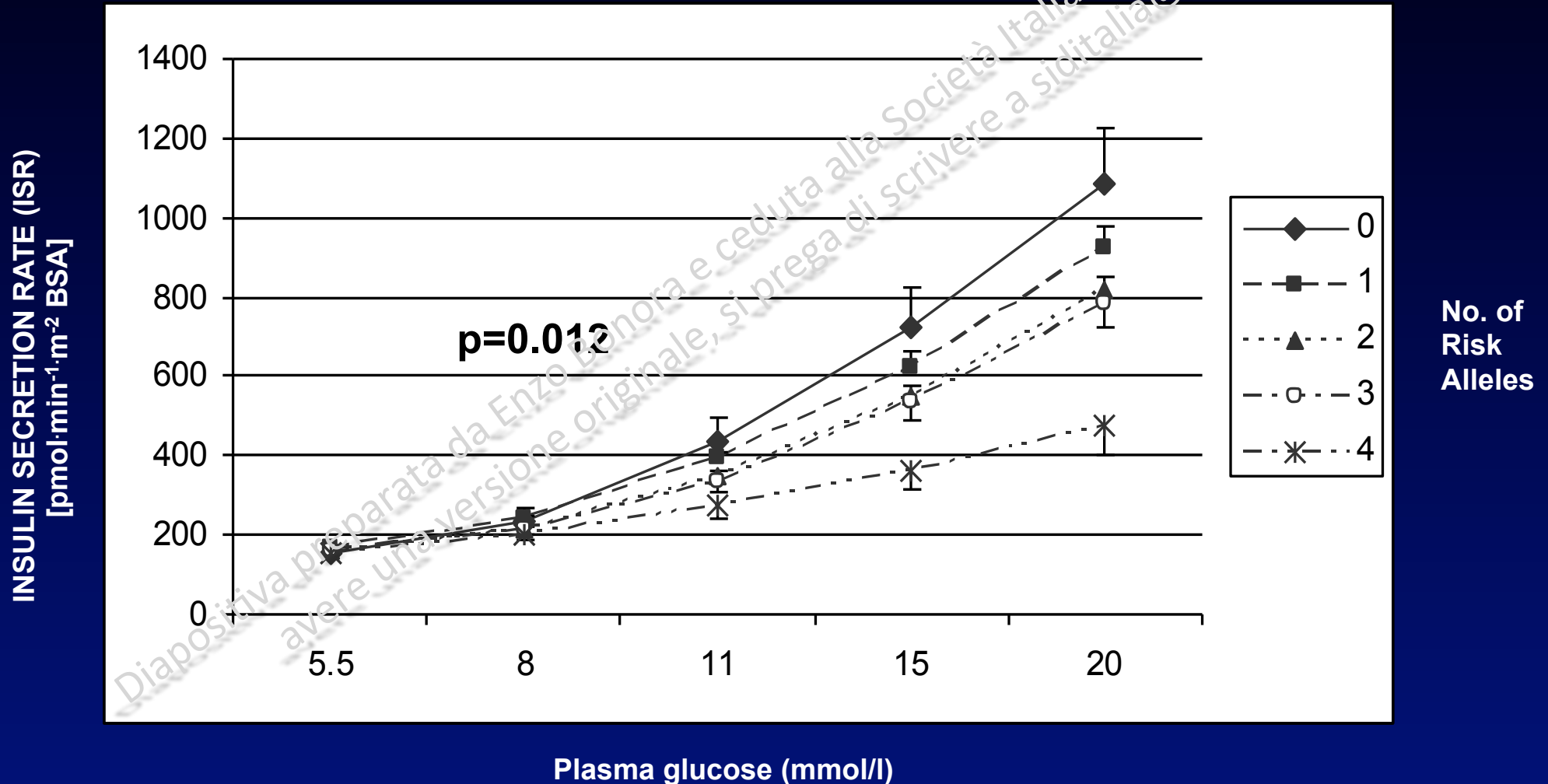
rs2384628



Plasma glucose (mmol/l)

Association of *GCKR* risk allele score with glucose stimulated insulin secretion in newly diagnosed T2DM

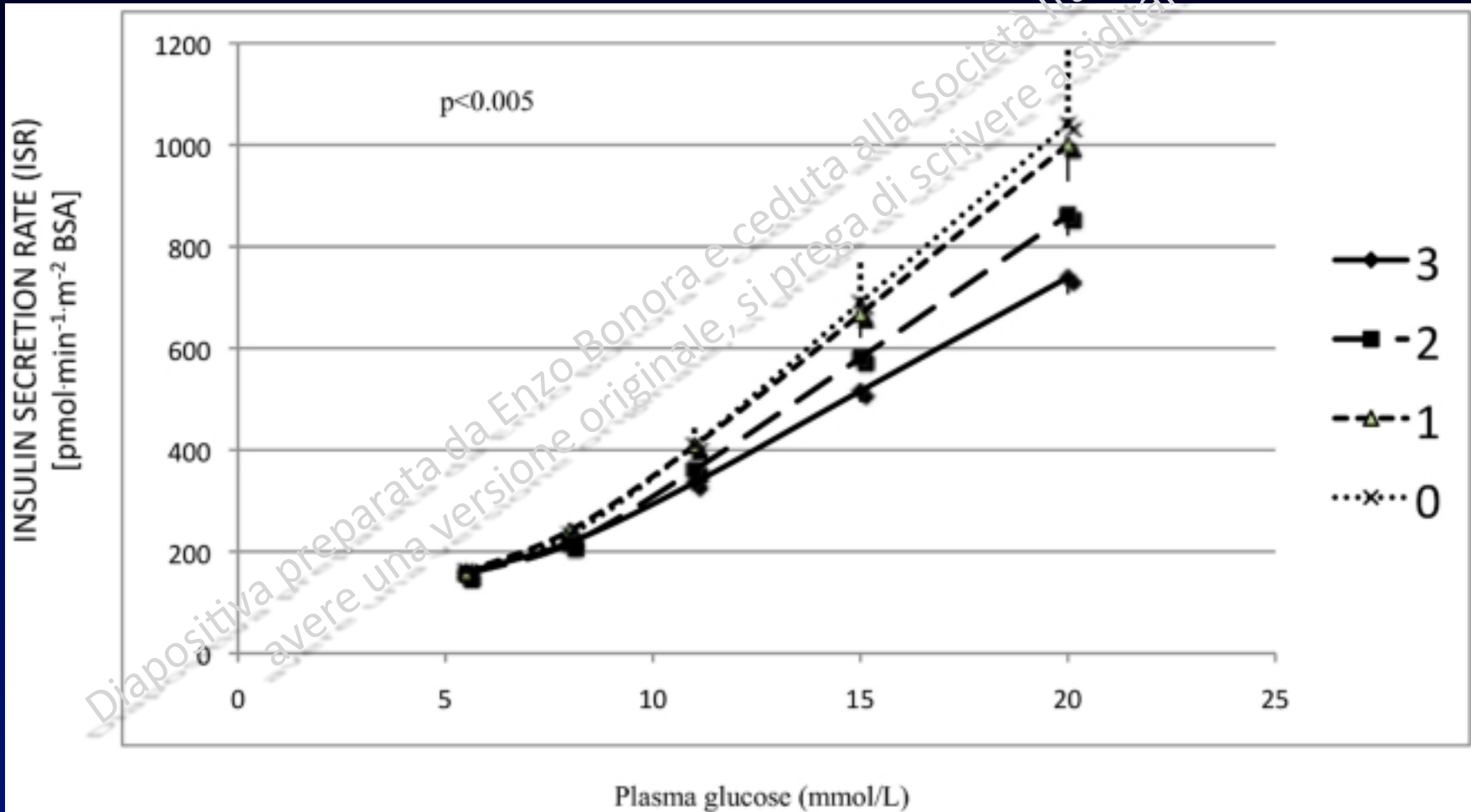
VNDS - Bonetti et al – Diabetes Care 2011



Association of *CACNA1E* risk allele score with glucose stimulated insulin secretion in newly diagnosed T2DM

VNDS – Trombetta et al – PLoSOne 7:e32755

CACNA1E = major subunit of the voltage-dependent Ca^{2+} channel $\text{Ca}_v2.3$



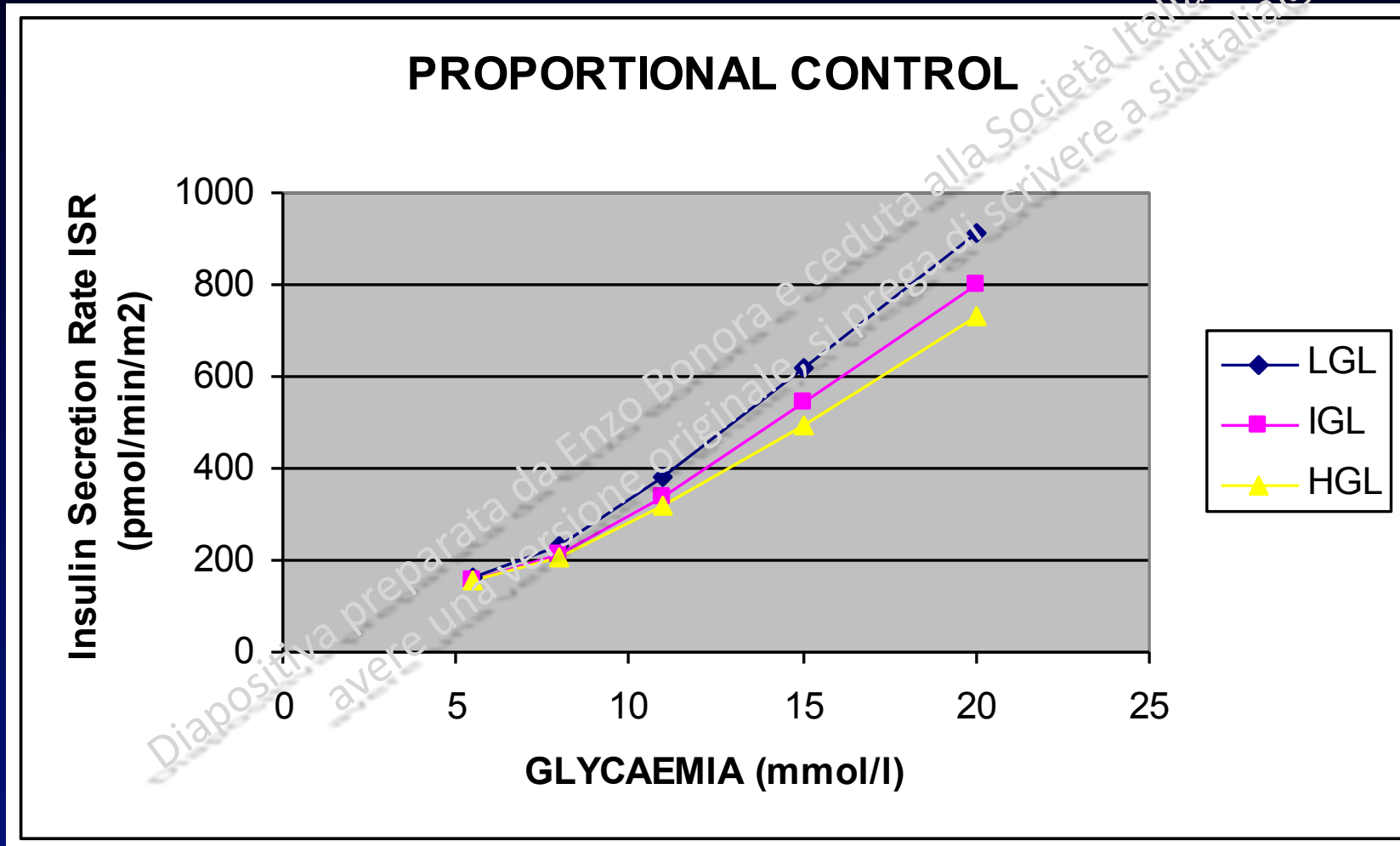
Genetic load assessment: 8 SNPs - 0 to 16 risk alleles

SNP	Nearest gene	Not-risk/risk alleles	MAF
rs679931	CACNA1E	A/T	0.37
rs1801282	PPARG	T/C	0.07
rs1044498	ENPP1	A/C	0.16
rs10946398	CDKAK1	A/C	0.30
rs1111875	HHEX	G/A	0.38
rs10010131	WFS1	A/G	0.35
rs4430796	TCF2	A/G	0.43
rs4402960	IGF2BP2	G/T	0.35

We gave different values to the alleles for each SNP: **0** to the not-risk allele and **1** to the risk allele. In this way we were able to calculate a sort of “genetic score”, summing up all the risk alleles values for each patients. Since we considered 8 SNPs, the genetic load score potentially ranges between 0 (patients with no risk alleles) and 16 (patients homozygous for risk alleles in all SNPs). We then divided the studied population into three groups on the basis of the number of risk alleles: **Low** (LGL: ≤ 6 , n=76), **Intermediate** (IGL: 7-9, n=226) and **High** (HGL: ≥ 10 , n=69) “Genetic Load” score.

Effect of “genetic load” on proportional control of insulin secretion in newly-diagnosed T2DM

VNDS - Bonetti et al – Diabetes Care 2011



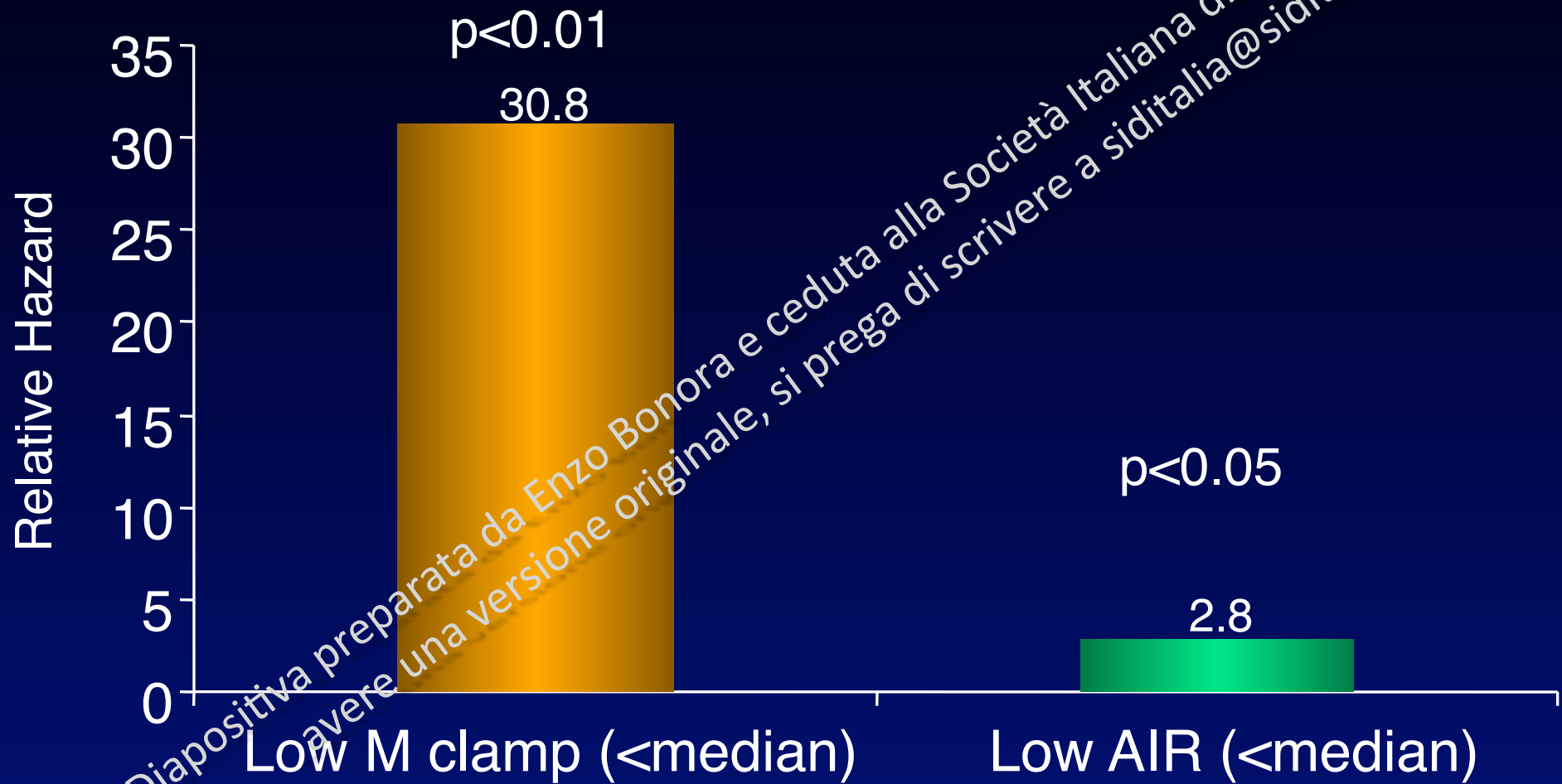
LGL: ≤ 6 , n=76
IGL: 7-9, n=226
HGL: ≥ 10 , n=69

Studi epidemiologici

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Risk of T2DM Associated to Insulin Resistance and Impaired Insulin Secretion in Pima Indians

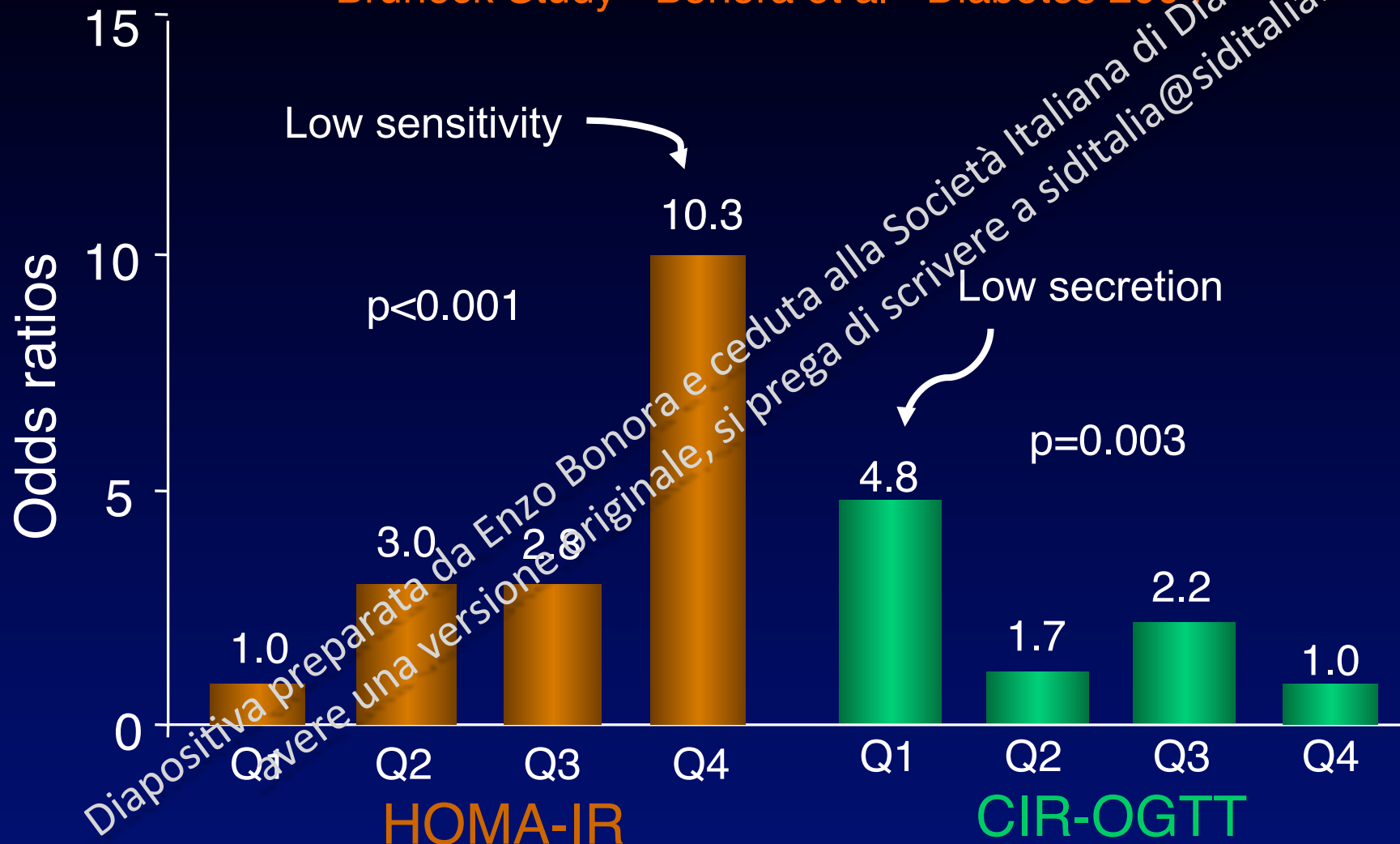
Lillioja et al - NEJM 1993; 329: 1988



N=200; follow-up 6 years; data are adjusted for sex, % body fat and WTR

Risk of Type 2 Diabetes According to Insulin Sensitivity and Insulin Secretion in Caucasians

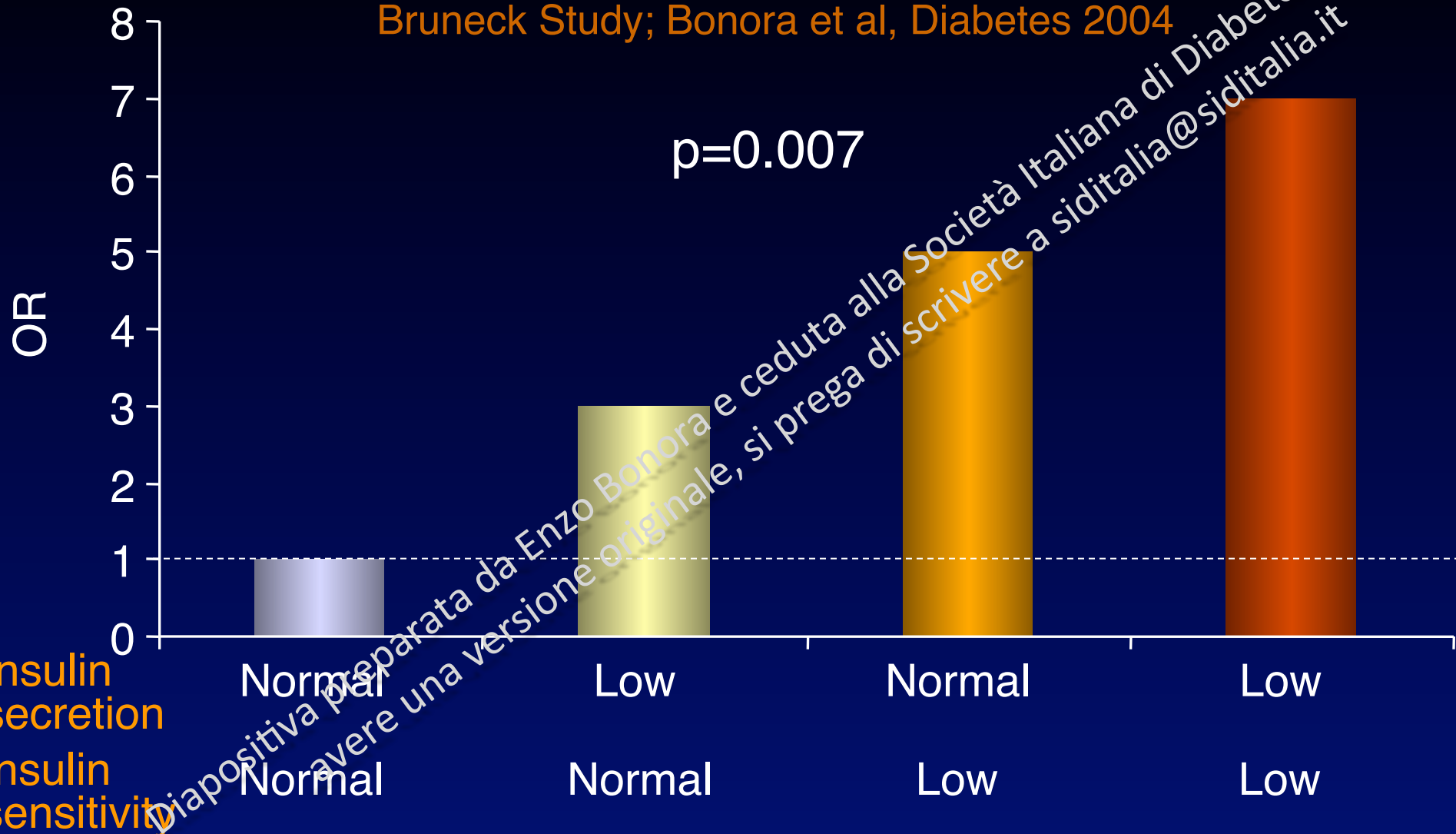
Bruneck Study - Bonora et al - Diabetes 2004



N=888; follow-up 10 years; data are adjusted for sex and age

HOMA-IR, CIR Index and 10-yr Incidence of T2DM in the General Population Aged 40-79 yr

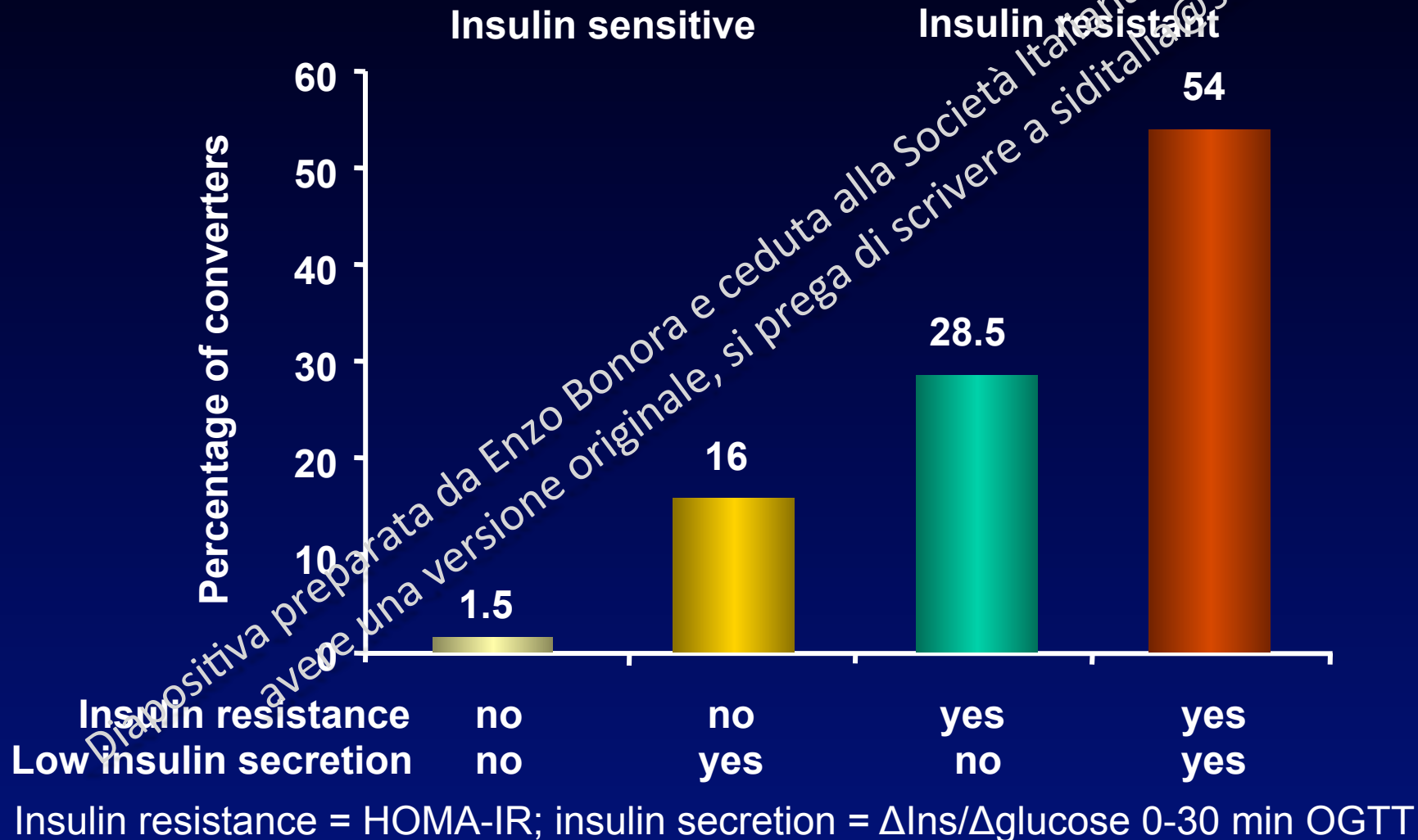
Bruneck Study; Bonora et al, Diabetes 2004



sex and age-adjusted

Incidence of type 2 diabetes according to insulin resistance and insulin secretion at baseline

Haffner et al, Circulation 101:975, 2000

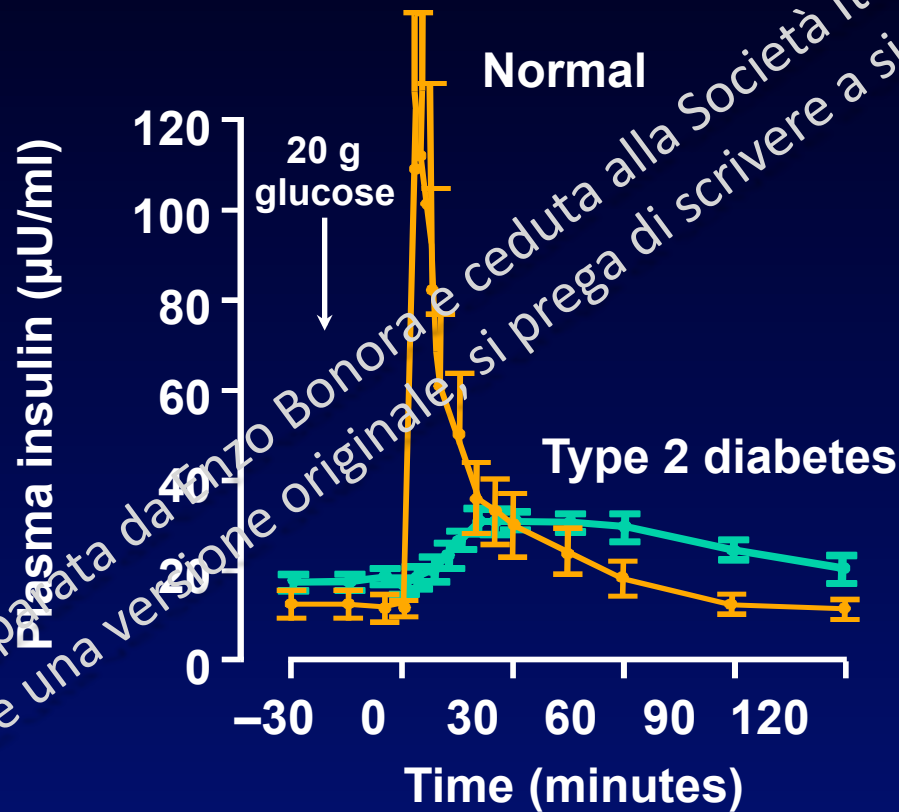


Studi di fisiopatologia clinica

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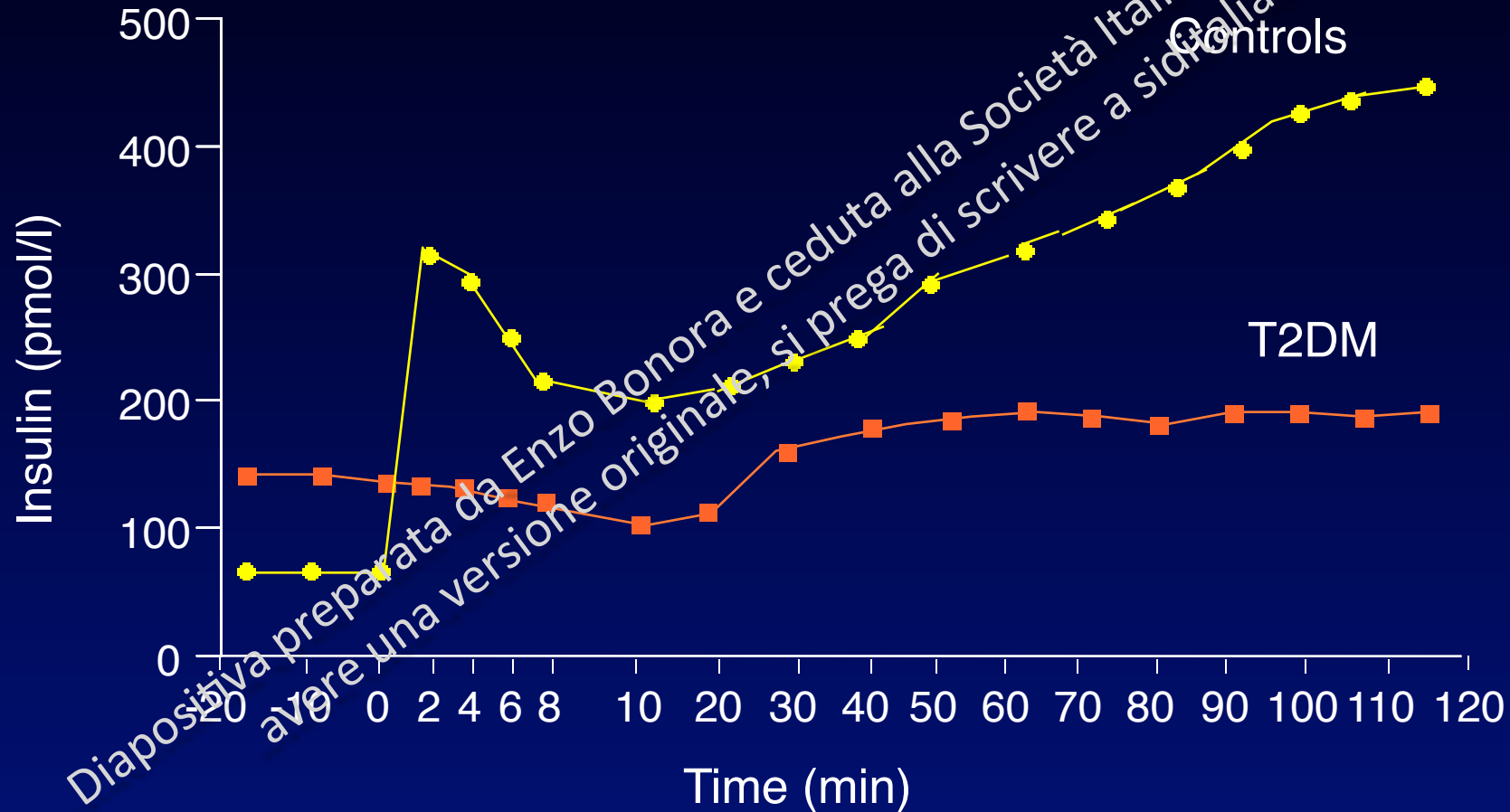
Loss of First-Phase Insulin Secretion after i.v. Glucose in T2DM

Ward WK et al - Diabetes Care 1984; 7:491



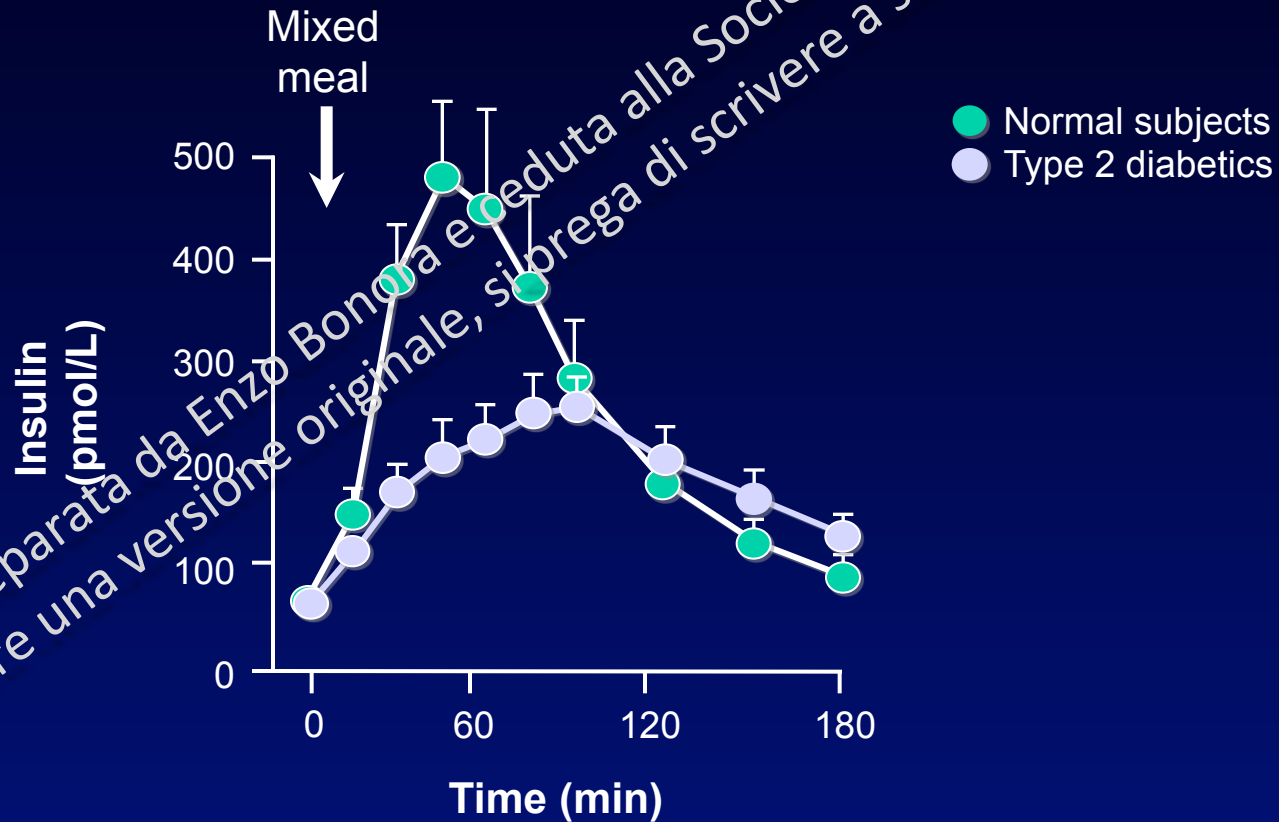
Impaired Insulin Response during Hyperglycemic Clamp in T2DM

Bonora et al; unpublished data



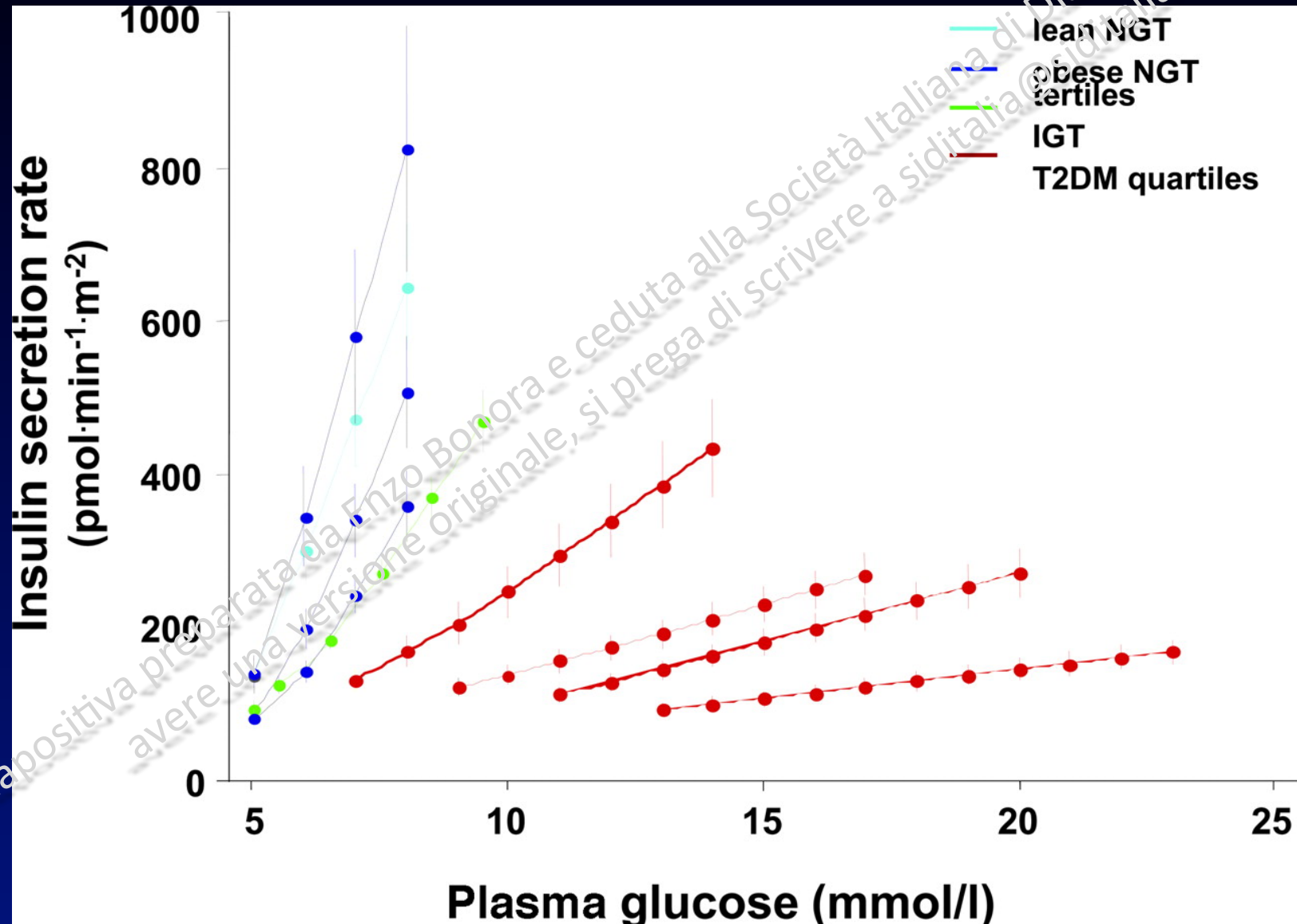
Impaired Insulin Response to Mixed Meal in T2DM

VilSBøll T et al - Diabetes 2001; 50: 609–613



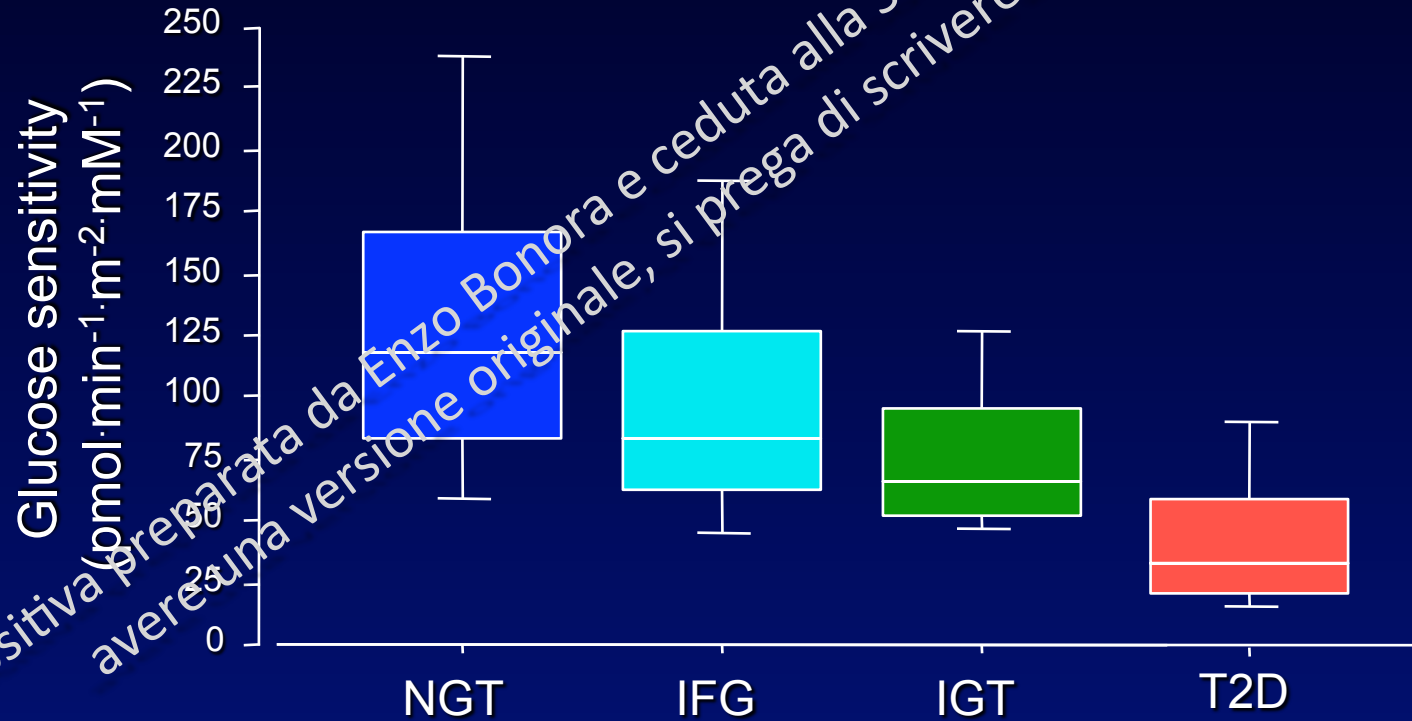
Dose-Response Curves for Glucose-Stimulated Insulin Secretion during OGTT in Different Conditions

Ferrannini et al - JCEM 2005; 90: 493



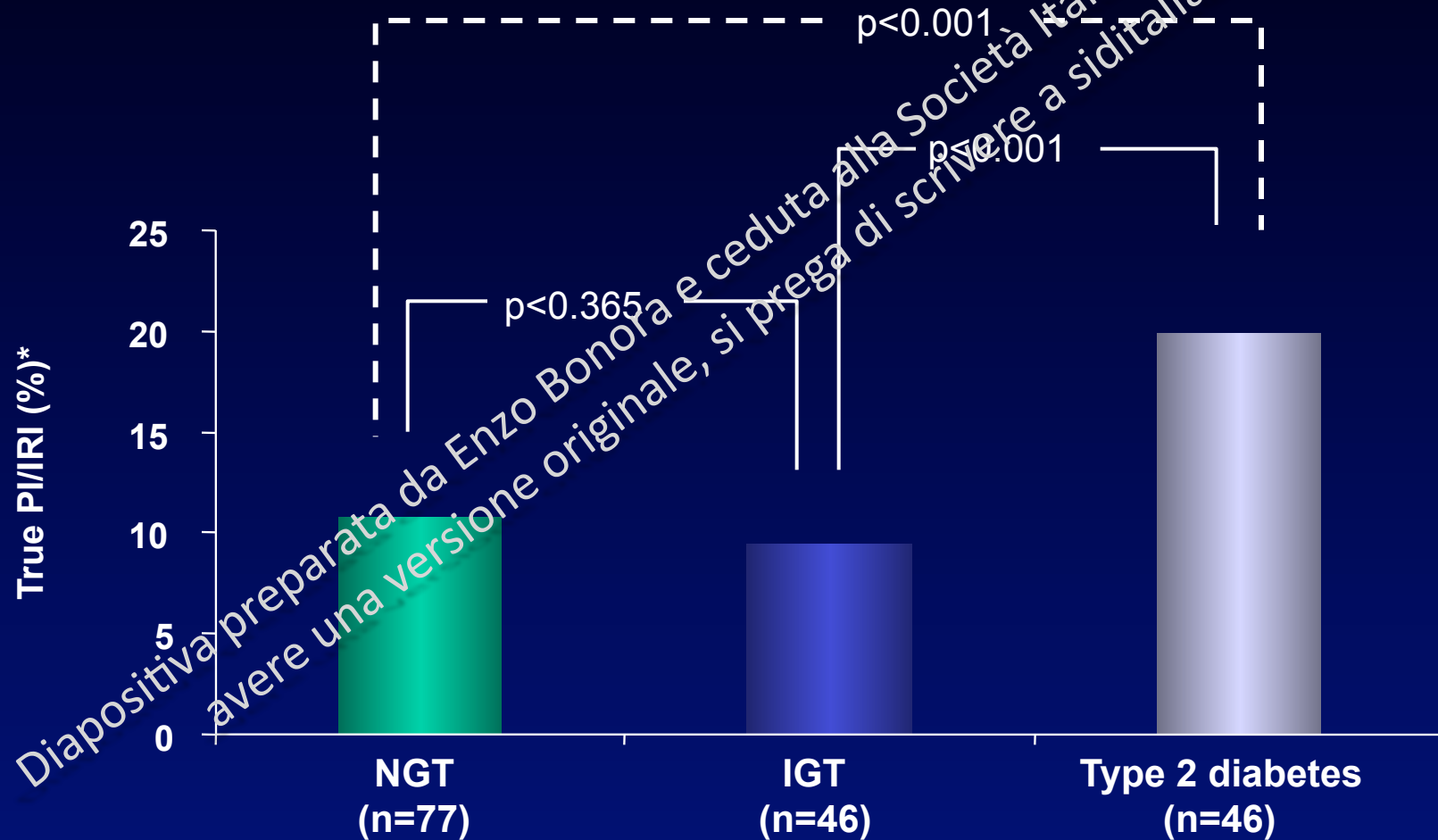
Beta-cell glucose sensitivity in T2DM according to glucose tolerance

Ferrannini E et al. Med Clin North Am 2011;95:227-39



Increased Ratio of Fasting Proinsulin to Insulin Ratio in T2DM

Saad et al, J Clin Endocrinol Metab 70: 1247–1253, 1990



Pulsatility of Insulin and Glucagon Secretion is Disrupted in the Fasting State and After Meal in T2DM

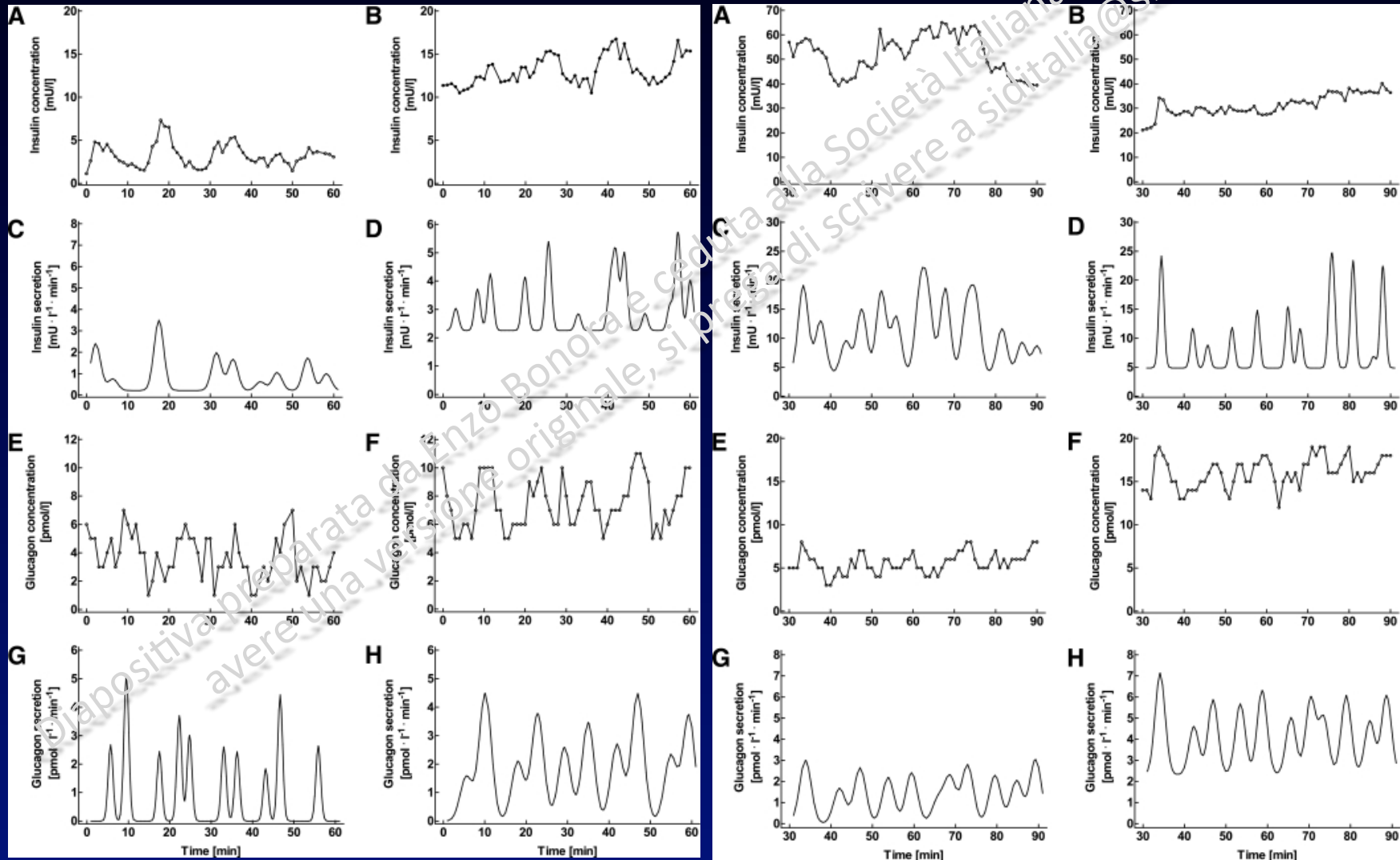
Menge BA et al – Diabetes 2011; 60: 2160-2168

Non diabetic

Diabetic

Non diabetic

Diabetic

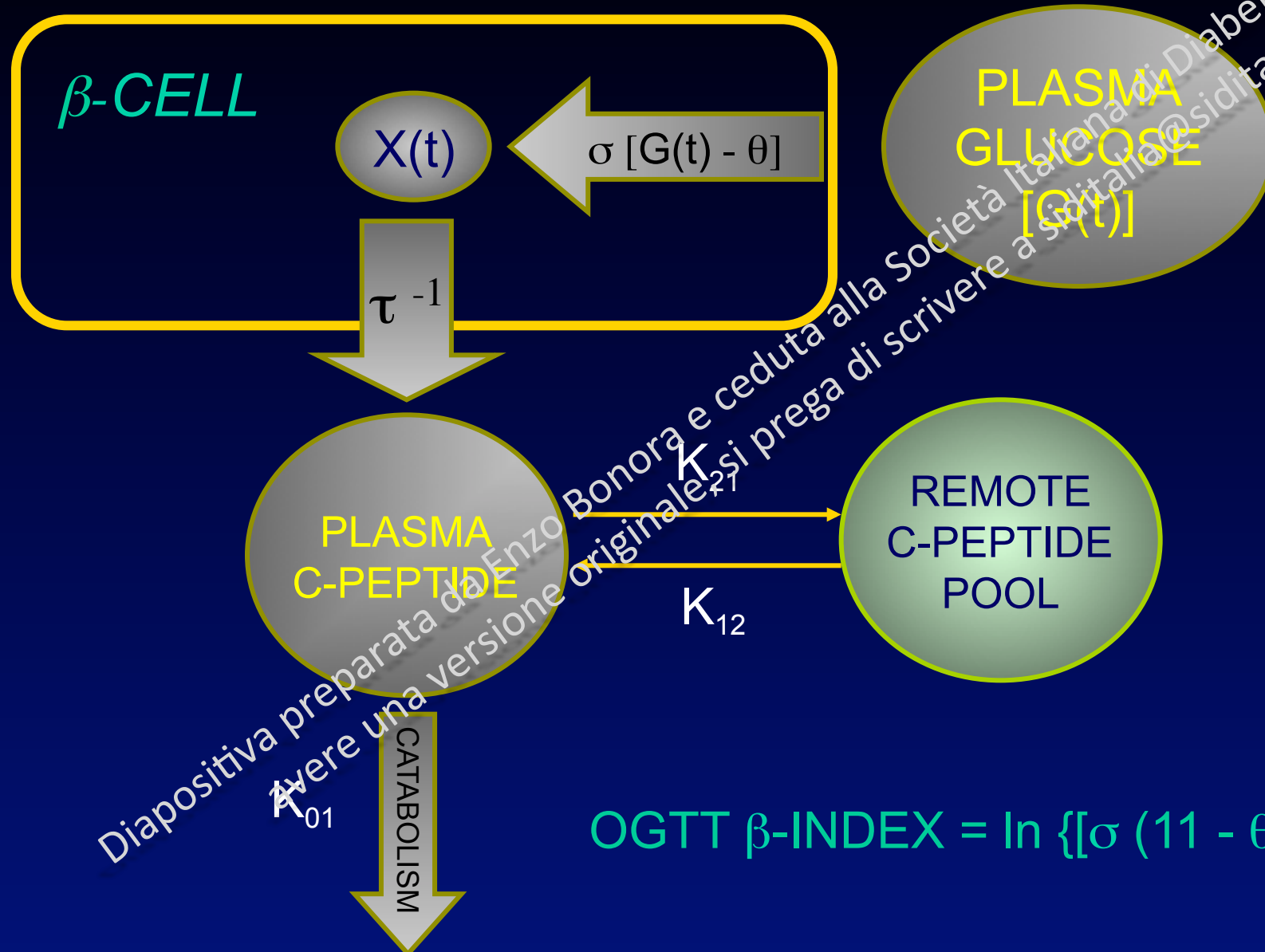


Fasting

Post-prandial

Minimal Model of β -Cell Function During OGTT

Cretti et al - Eur J Clin Invest 2001; 31:405



$$\text{OGTT } \beta\text{-INDEX} = \ln \{ [\sigma (11 - \theta)] / (2.72 + \tau) \}$$

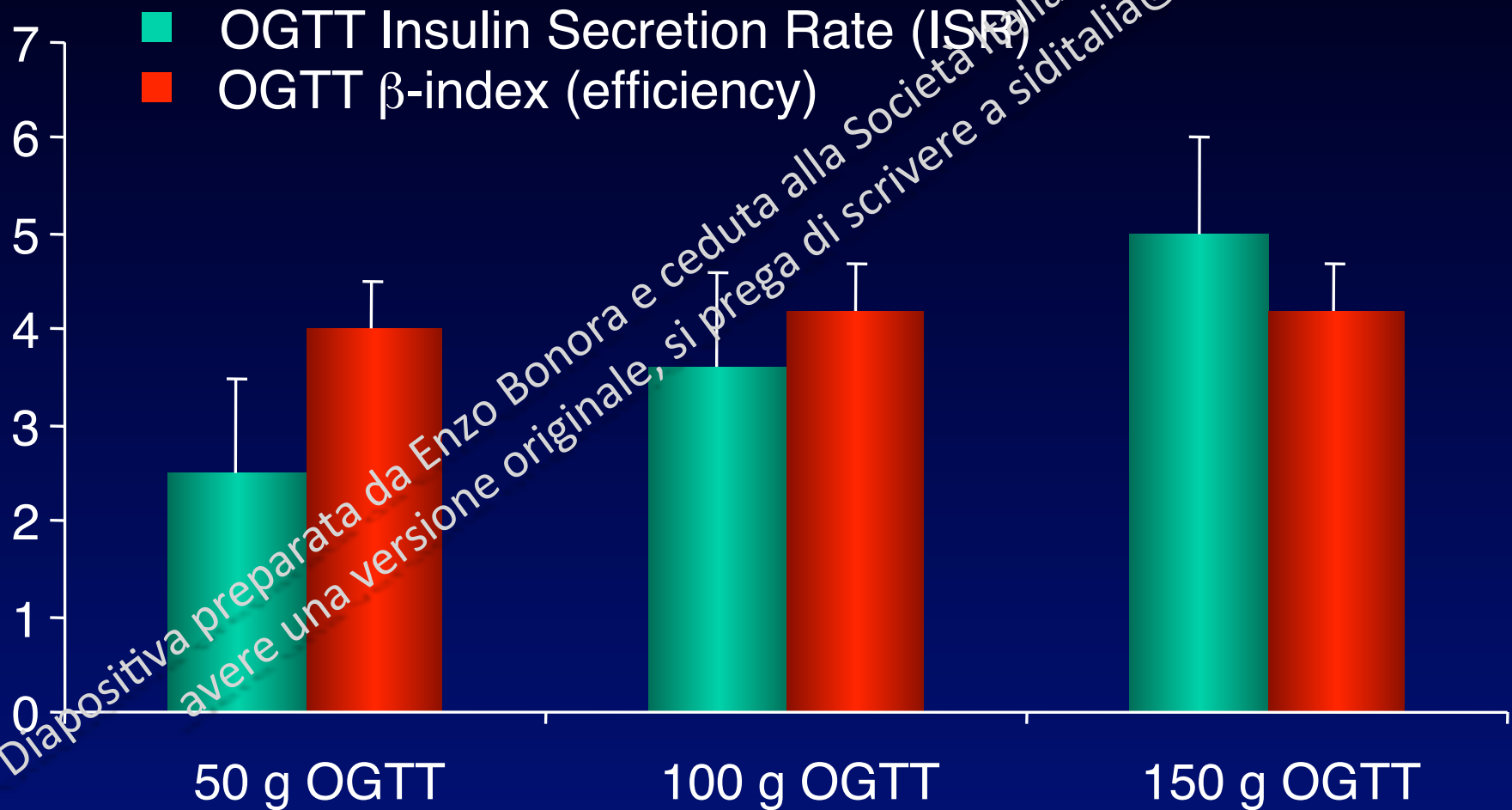
What does OGTT β -Index Represent?

- Secretory force of the beta-cell
- Beta-cell competence for glucose
- Efficiency of beta-cell

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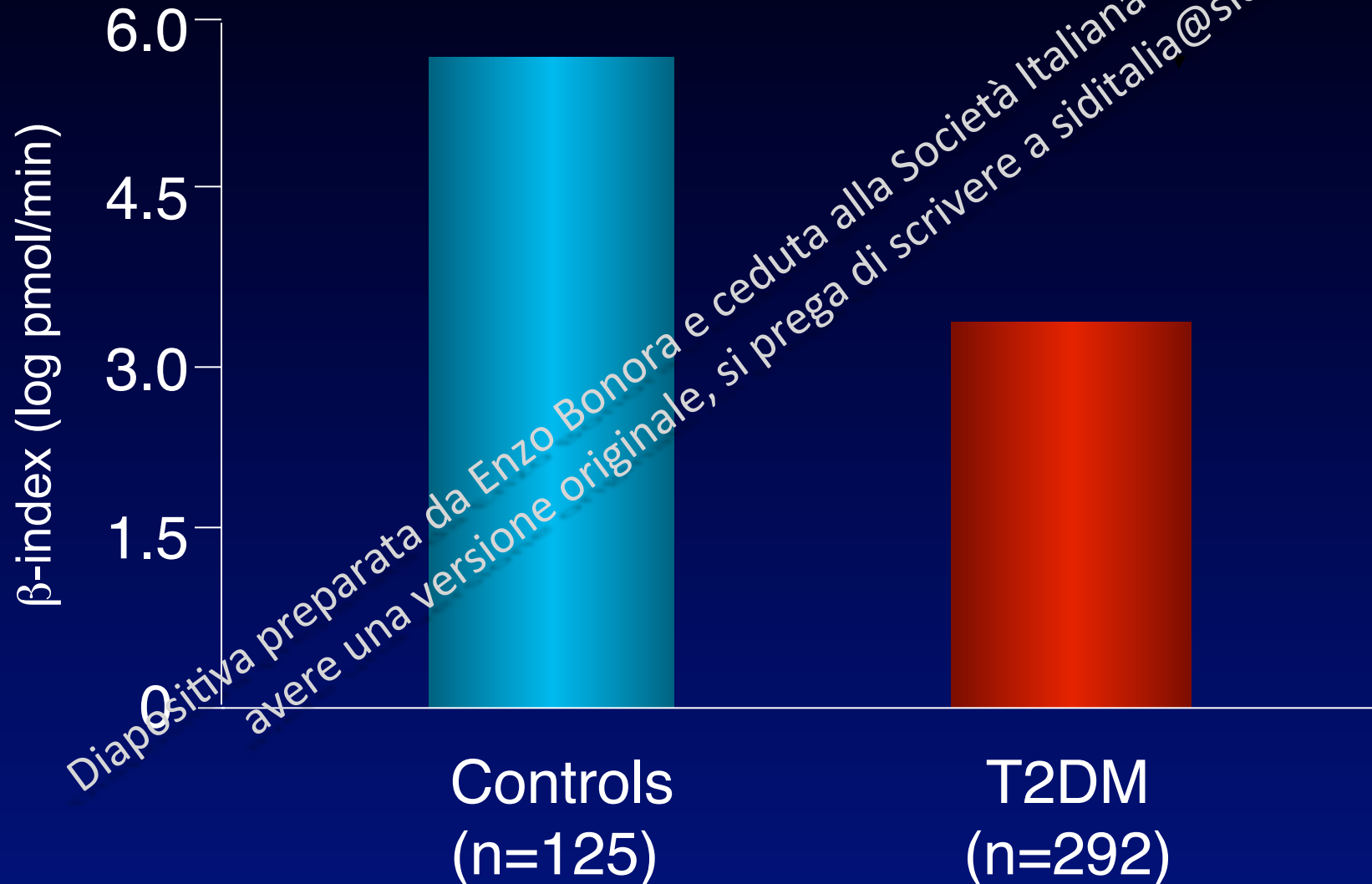
OGTT-ISR and β -Index Values during Oral Glucose Loads of 50, 100 and 150 g

Cretti et al - Eur J Clin Invest 2001; 31:405



Impaired Beta-cell Efficiency in Newly-Diagnosed T2DM

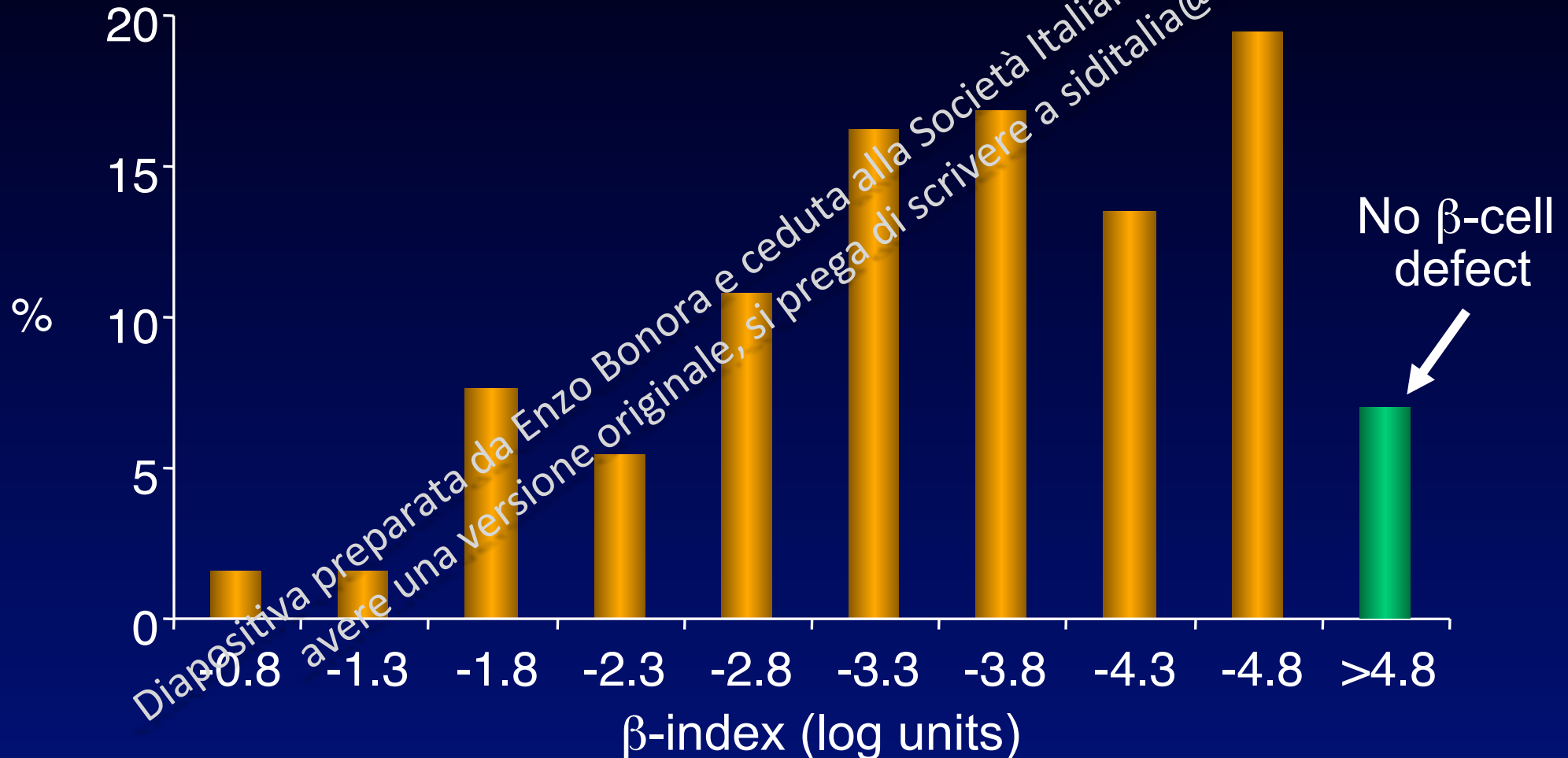
Bonora and Bonadonna - unpublished data



Frequency Distribution of β -Index in Subjects with Newly-Diagnosed T2DM

Bonora and Bonadonna - unpublished data

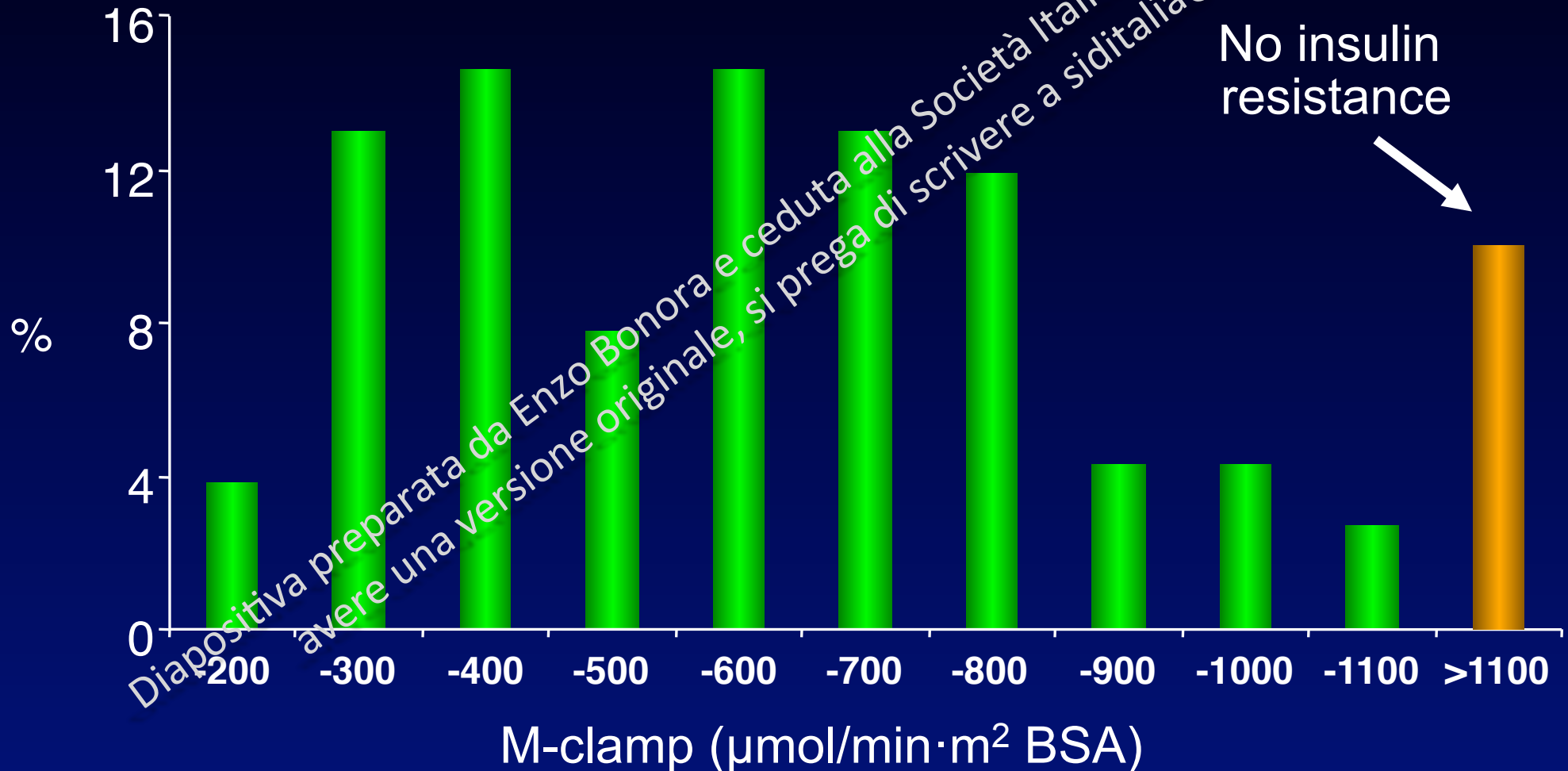
Threshold of β -cell defect = upper limit of bottom quintile of β -index in 125 non-obese healthy subjects



Frequency Distribution of M-clamp in Subjects with Newly-diagnosed T2DM

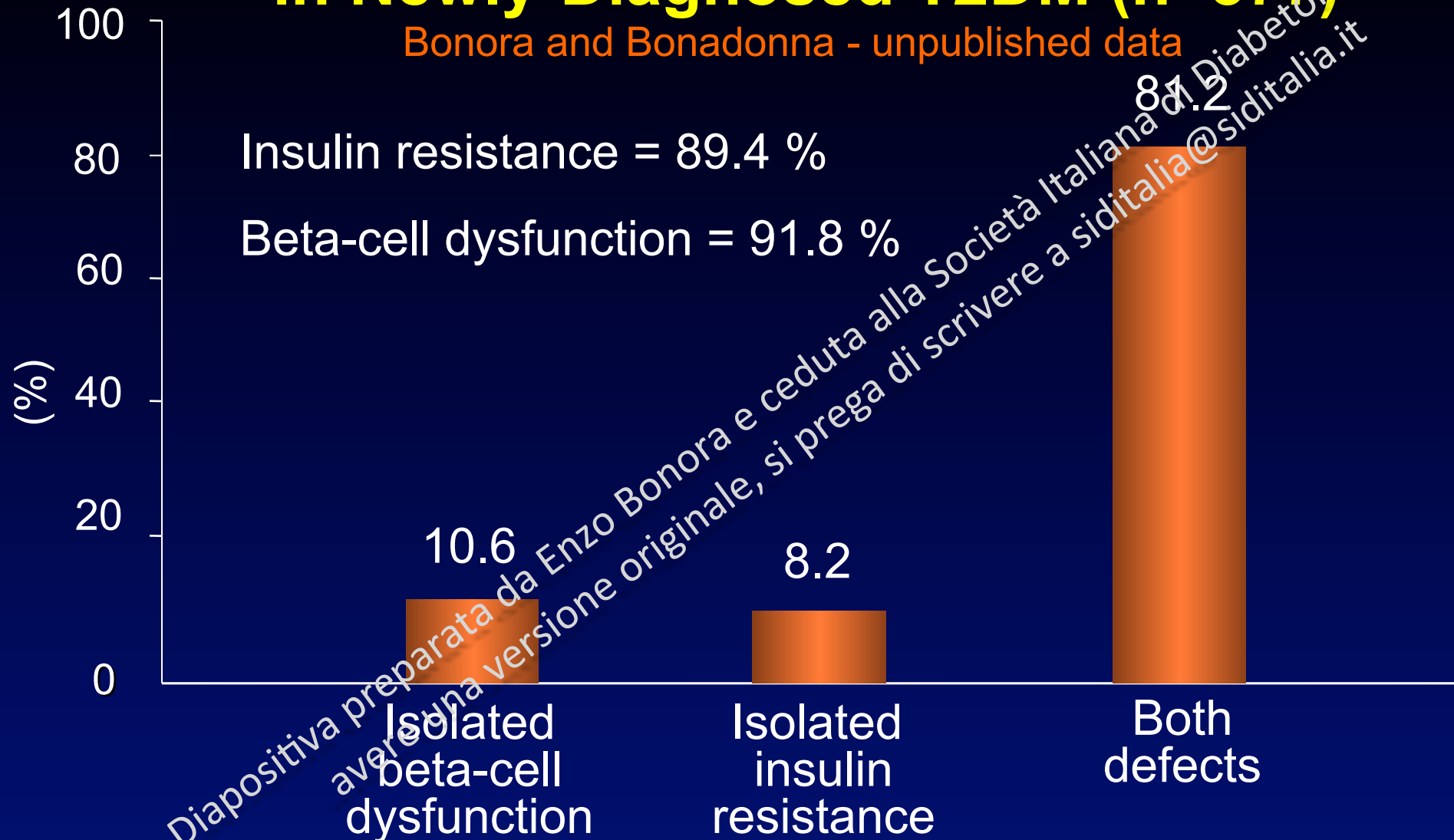
Bonora and Bonadonna - unpublished data

Threshold of insulin resistance = upper limit of bottom quintile of M-clamp in 515 non-obese healthy subjects



Proportion of Pathogenic Phenotypes in Newly-Diagnosed T2DM (n=577)

Bonora and Bonadonna - unpublished data

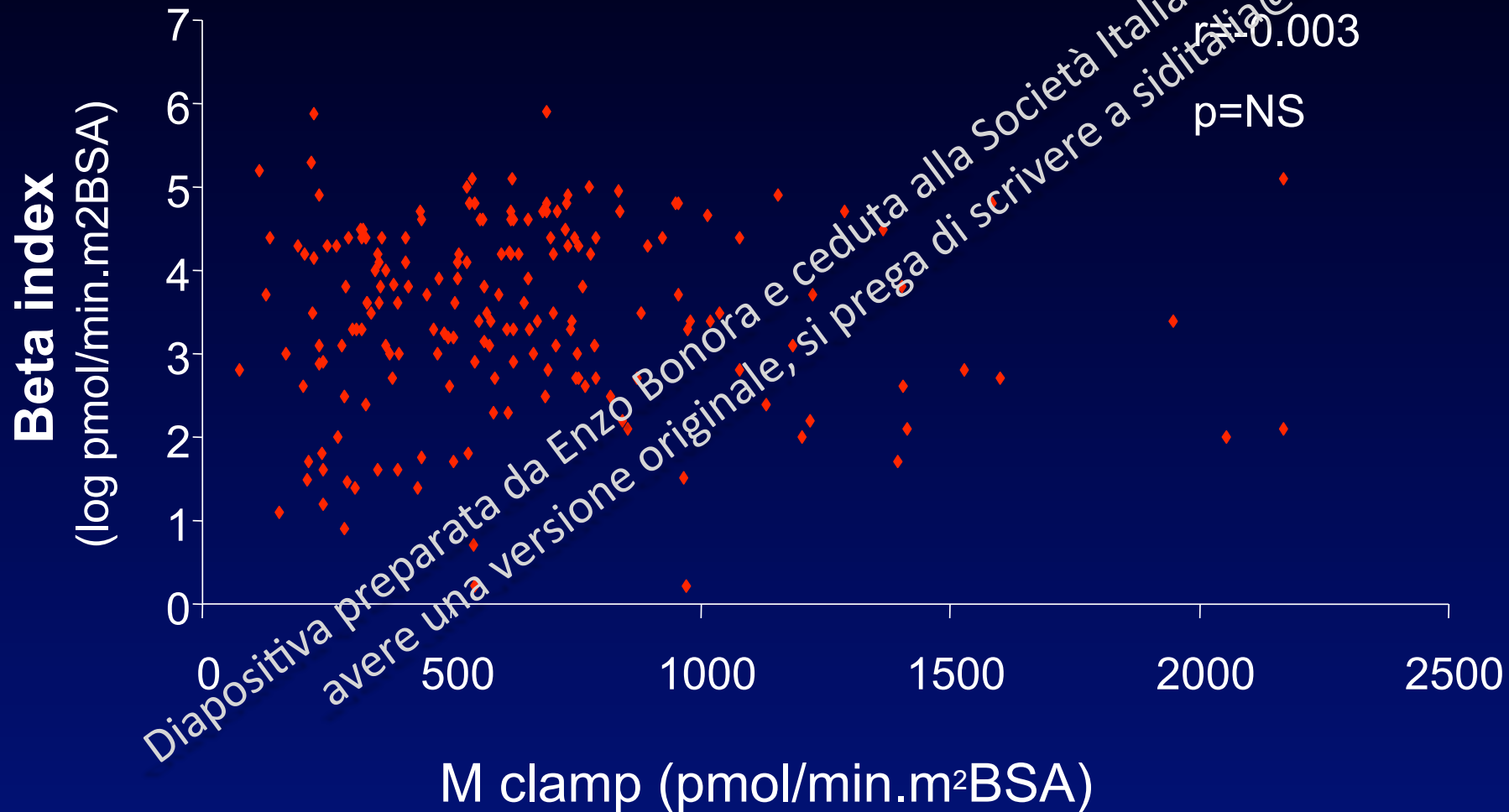


insulin sensitivity assessed by euglycemic clamp (M)

insulin secretion assessed by OGTT and minimal model (β -index)

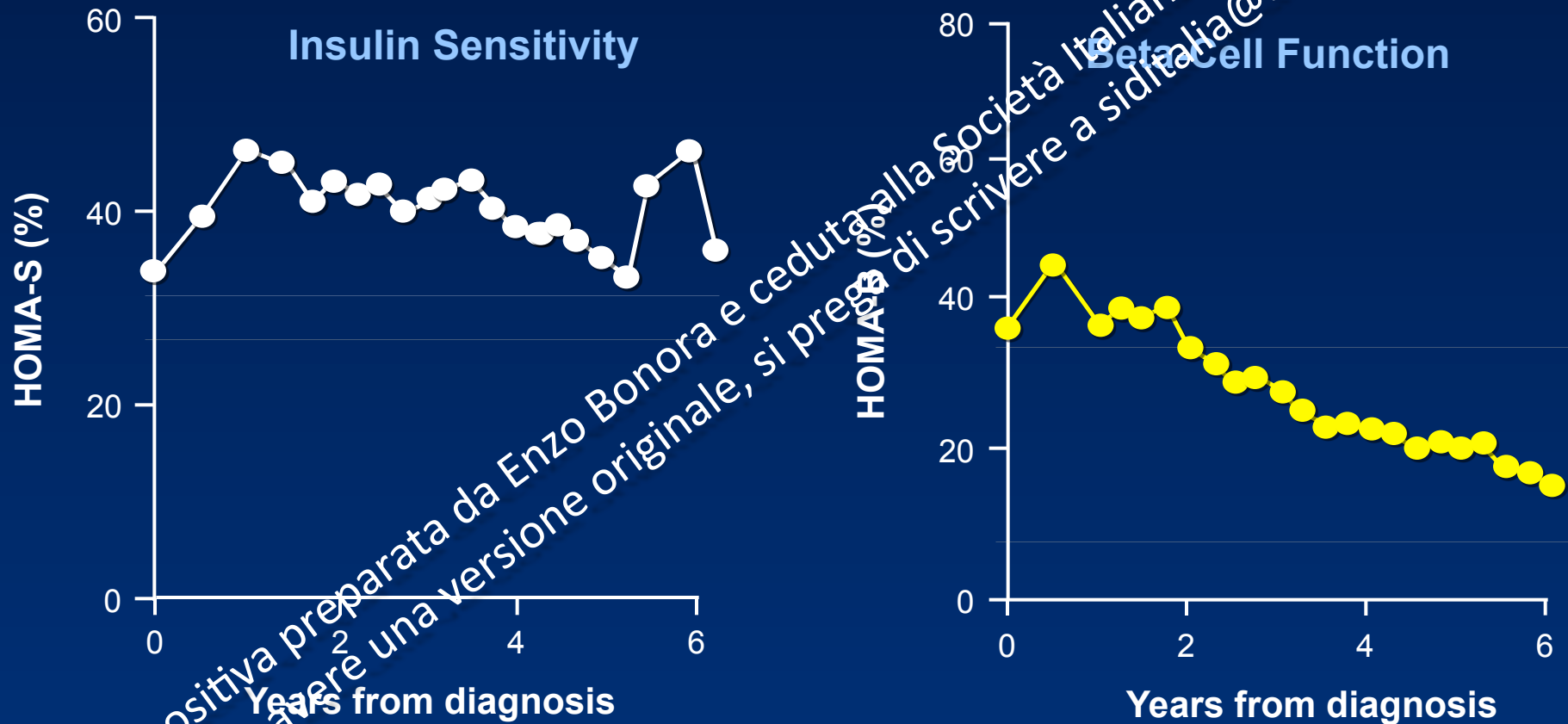
Relationship between M-clamp and β -Index in Newly-Diagnosed T2DM

Bonora et al, unpublished data



Beta-Cell Function Declines While Insulin Sensitivity Remains Stable in Type 2 Diabetes

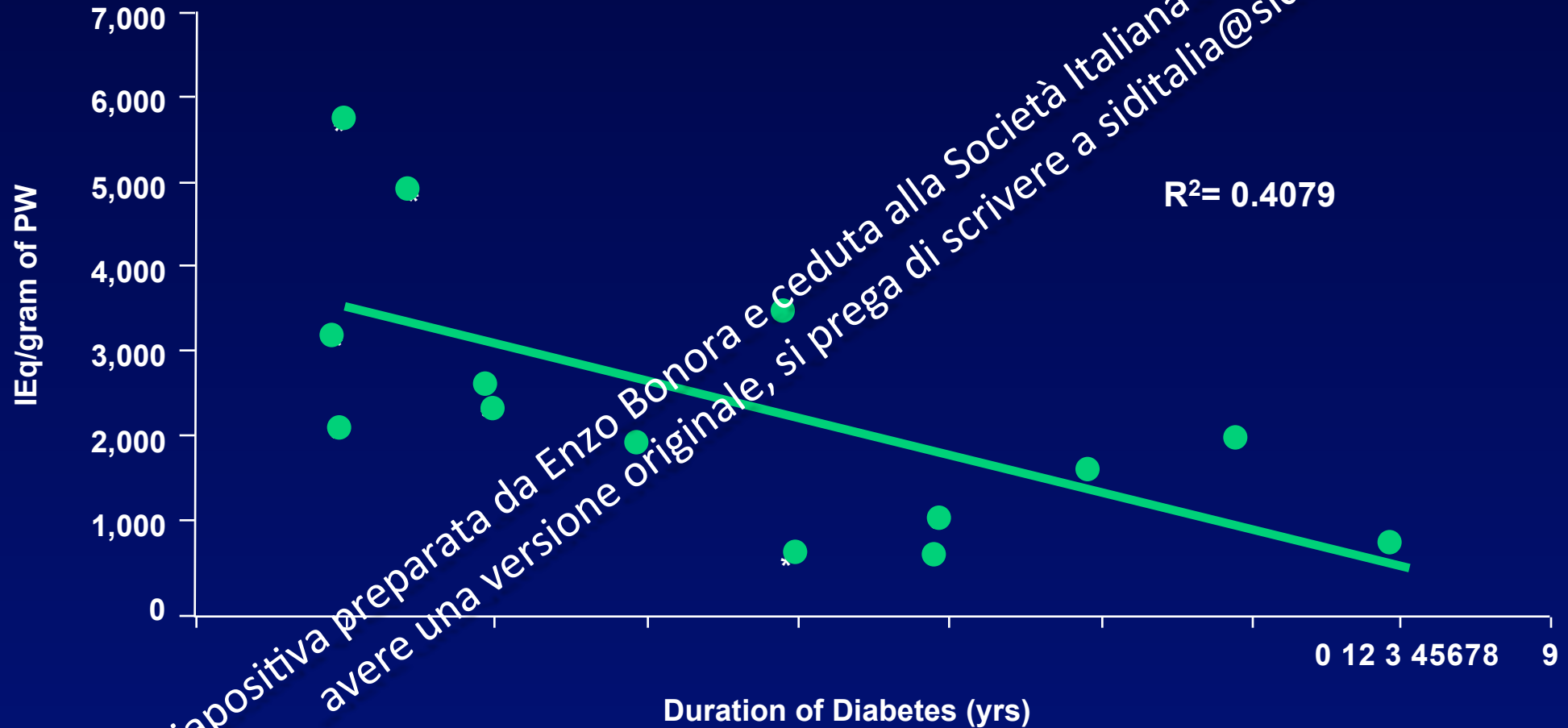
Levy et al - Diabet Med 1988; 15: 290



10-year follow-up of the Belfast Diet Study: Data from newly diagnosed subjects with type 2 diabetes mellitus (N=67) who required oral antihyperglycemic therapy or insulin due to secondary failure of diet therapy at 5–7 years.

Islets Mass Declines with Progression of T2DM

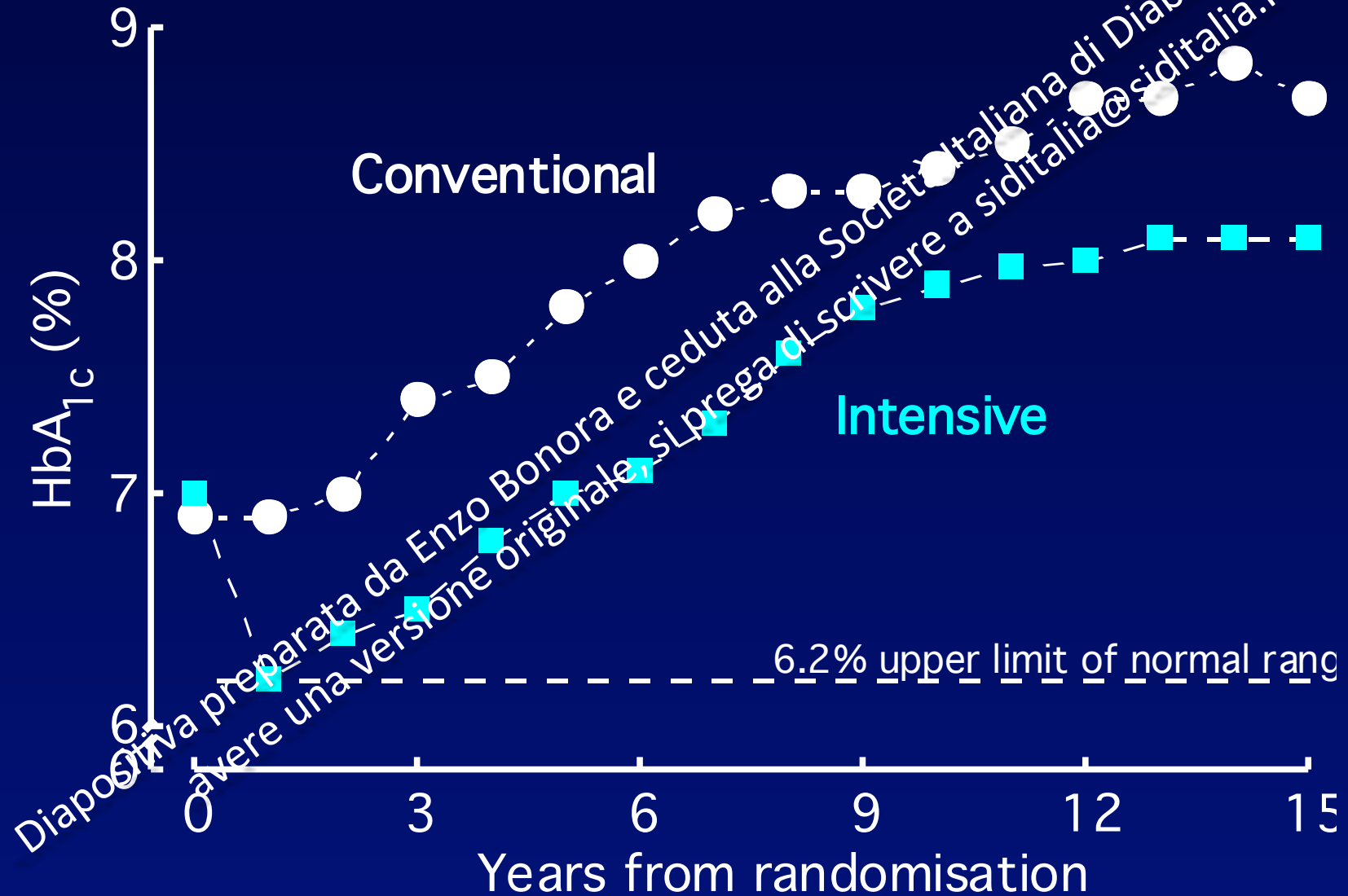
Deng et al - Diabetes 2004; 53: 624



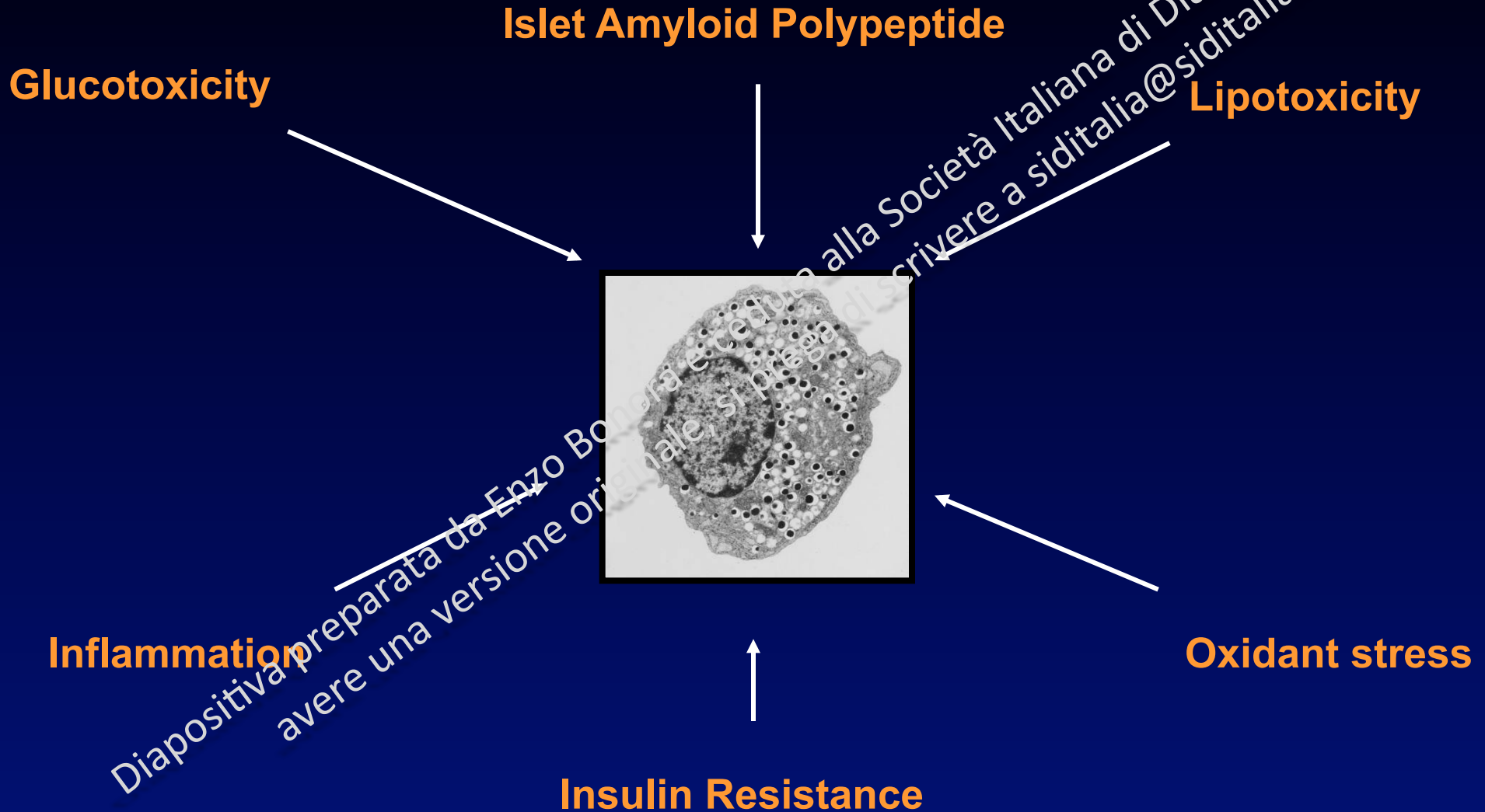
IEq= Islet Equivalents; PW= Pancreas Weight

United Kingdom Prospective Diabetes Study (UKPDS)

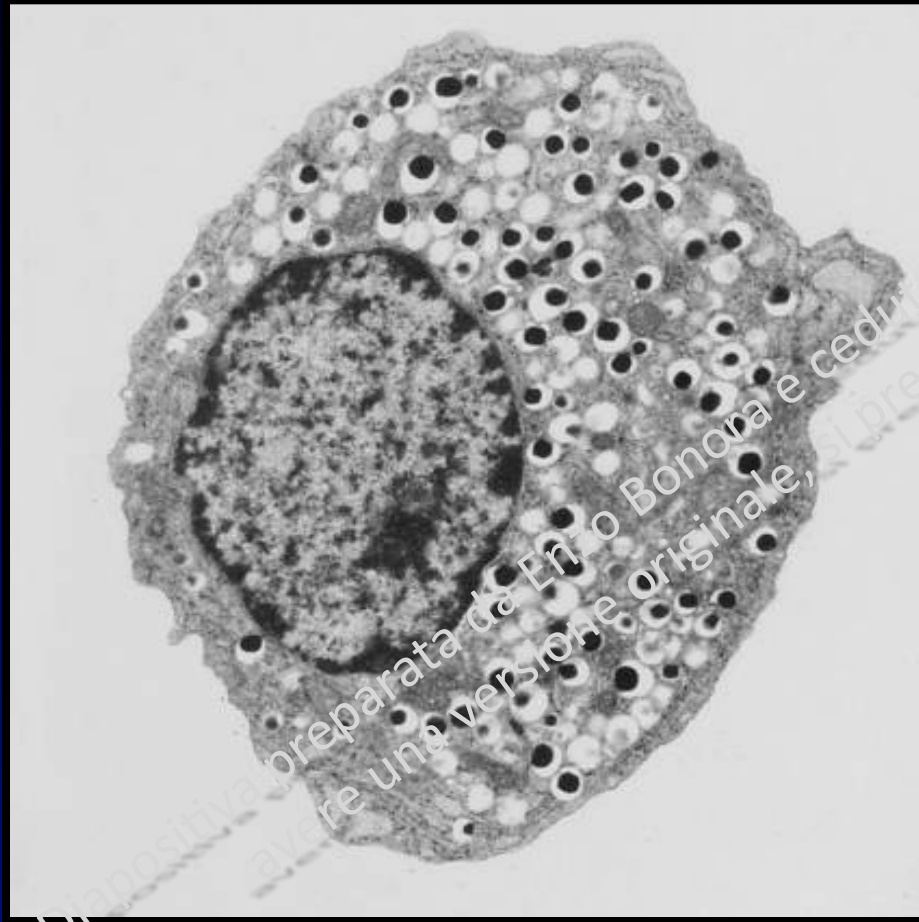
Progressive Loss of glucose control



Insults to the Beta-Cell



The Normal Beta-Cell

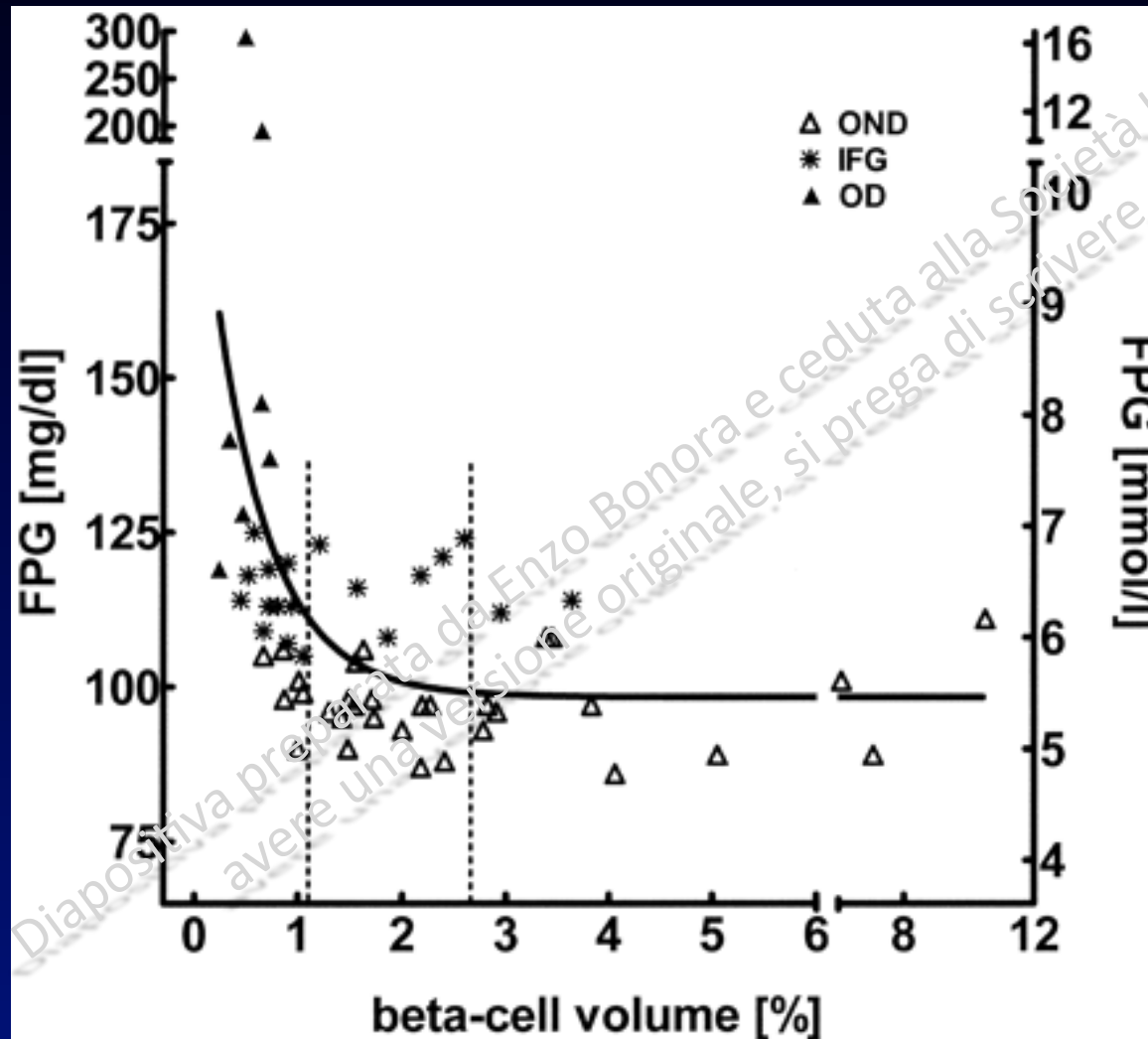


10 μm

~ 10,000
granules

Relationship of Fasting Plasma Glucose to Beta-Cell Volume in Pancreas from Untreated Obese Humans

Ritzel et al - Diabetes Care 2006; 29: 717

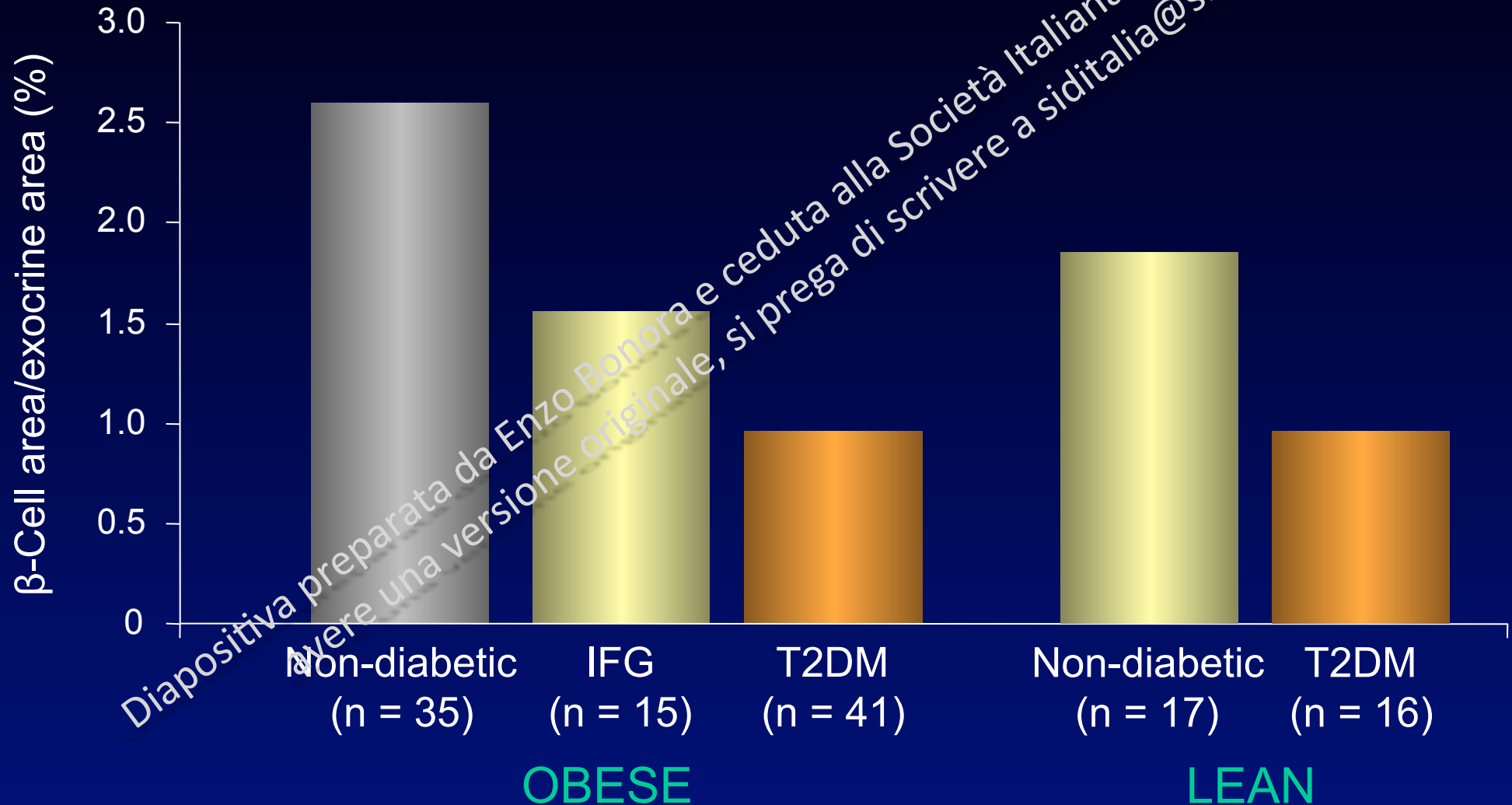


Monoesponential fit

$R=0.50$, $p<0.001$

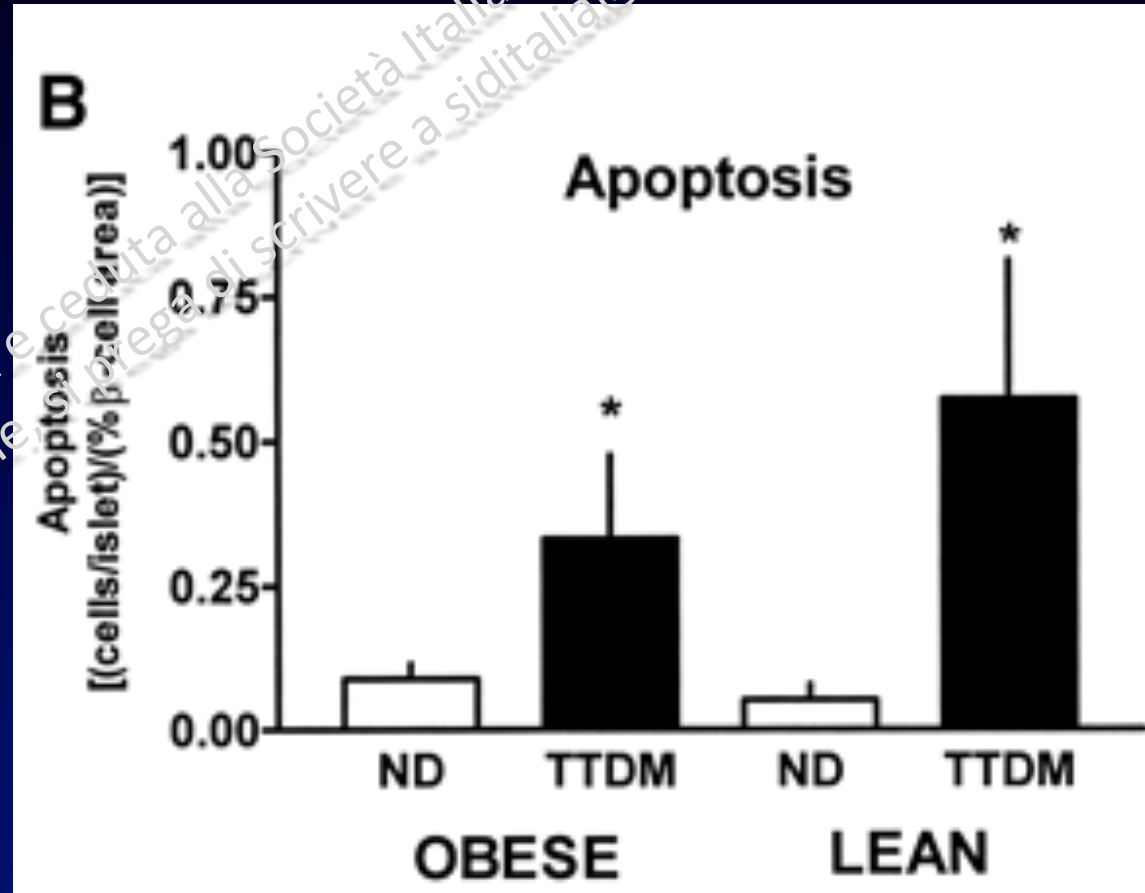
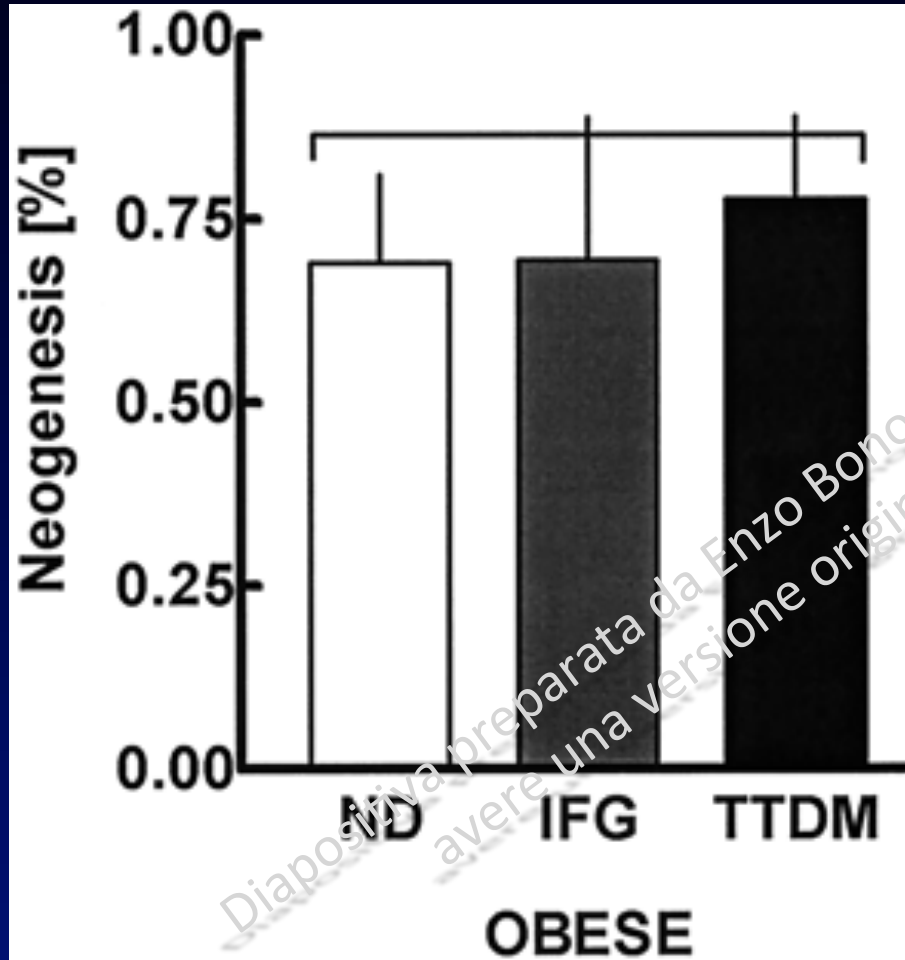
Beta-Cell Volume in Pancreas from Obese and Lean Subjects with IFG and T2DM

Butler et al - Diabetes 2003; 52:102



Apoptosis and Neogenesis of Beta-Cell in Pancreas from Nondiabetic and Diabetic subjects

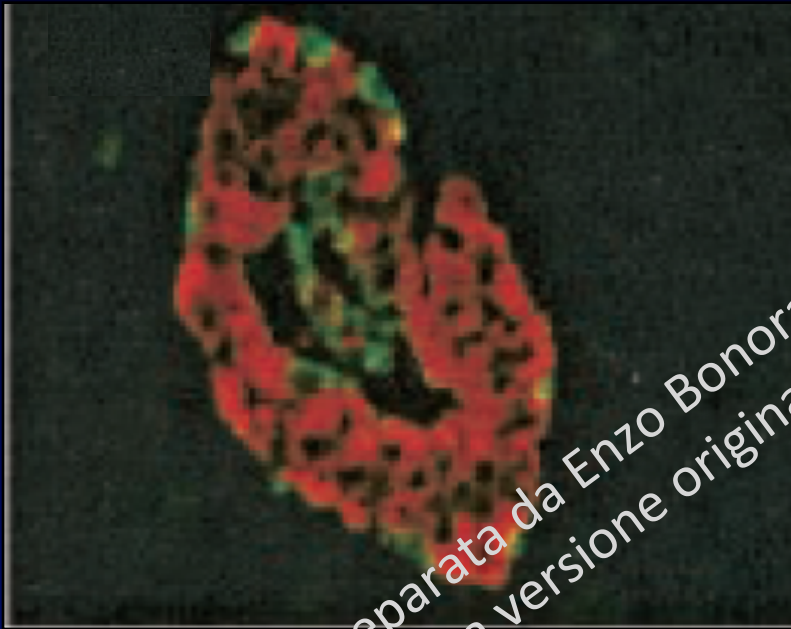
Butler et al - Diabetes 2003; 52:102



Beta-Cells are Reduced and Alpha-Cells are Increased in Human Pancreatic Islets from T2DM

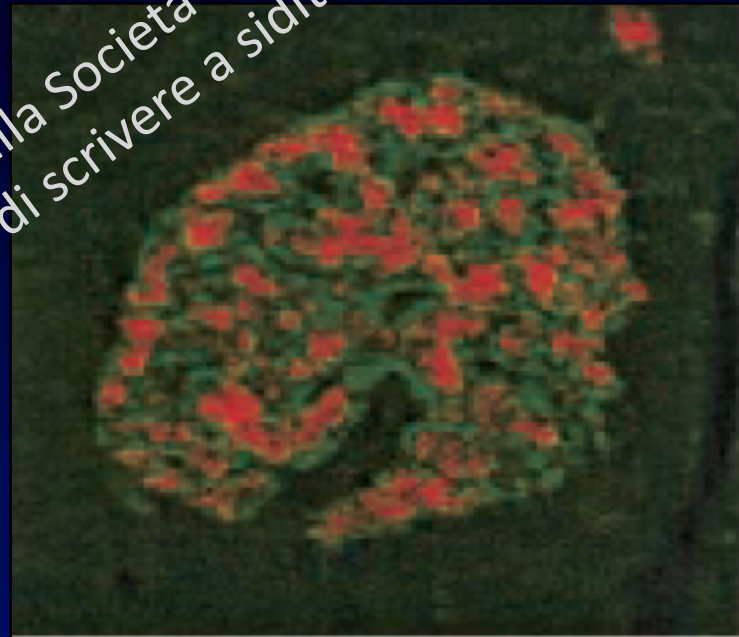
Deng et al; Diabetes 53: 624, 2004

Nondiabetic



33% α -cells
65% β -cells

T2DM



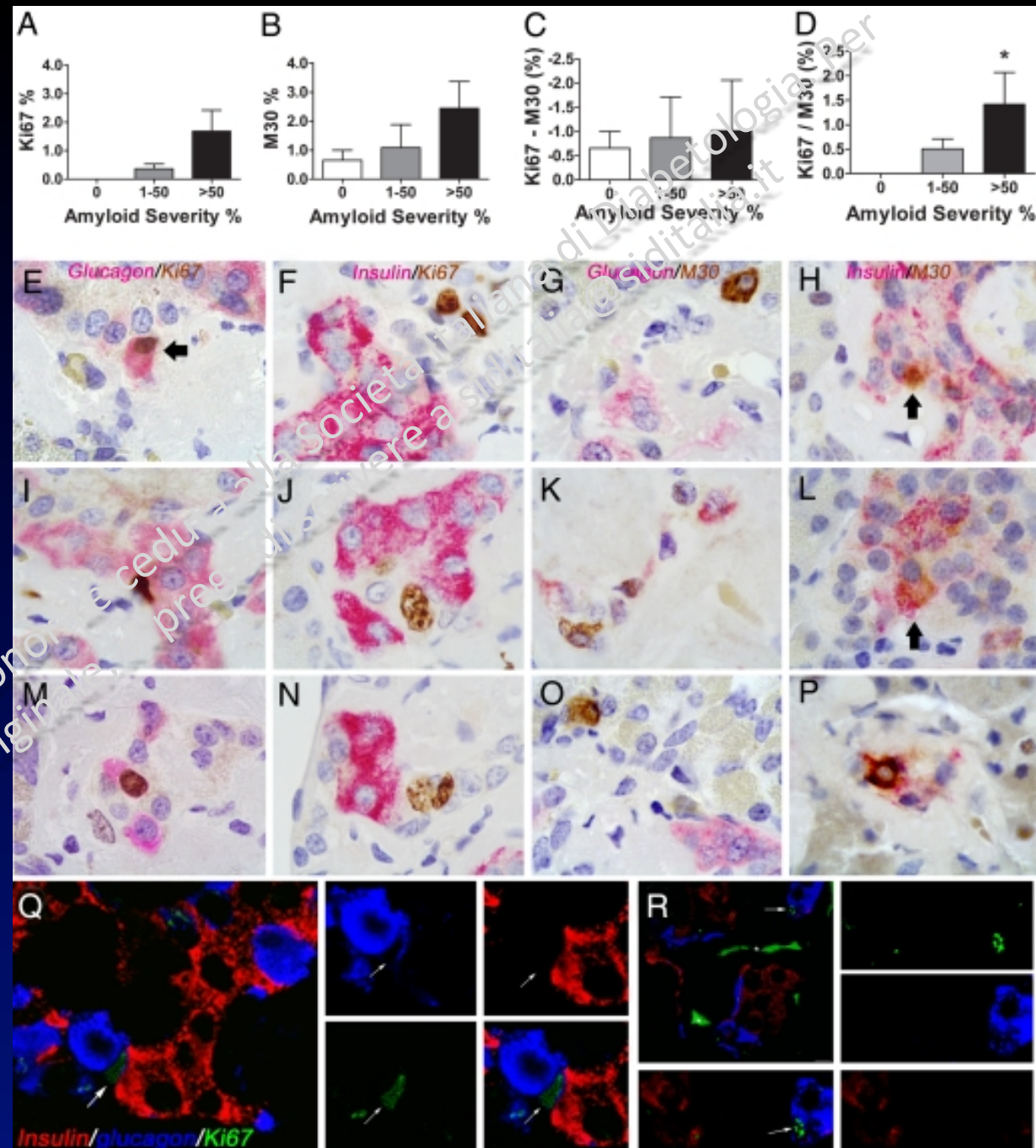
52% α -cells
48% β -cells

$P < 0.01$

Islets of Baboons with Type 2 Diabetes Have Increased Proliferation of Alpha-Cells and Increased Apoptosis of Beta-Cells

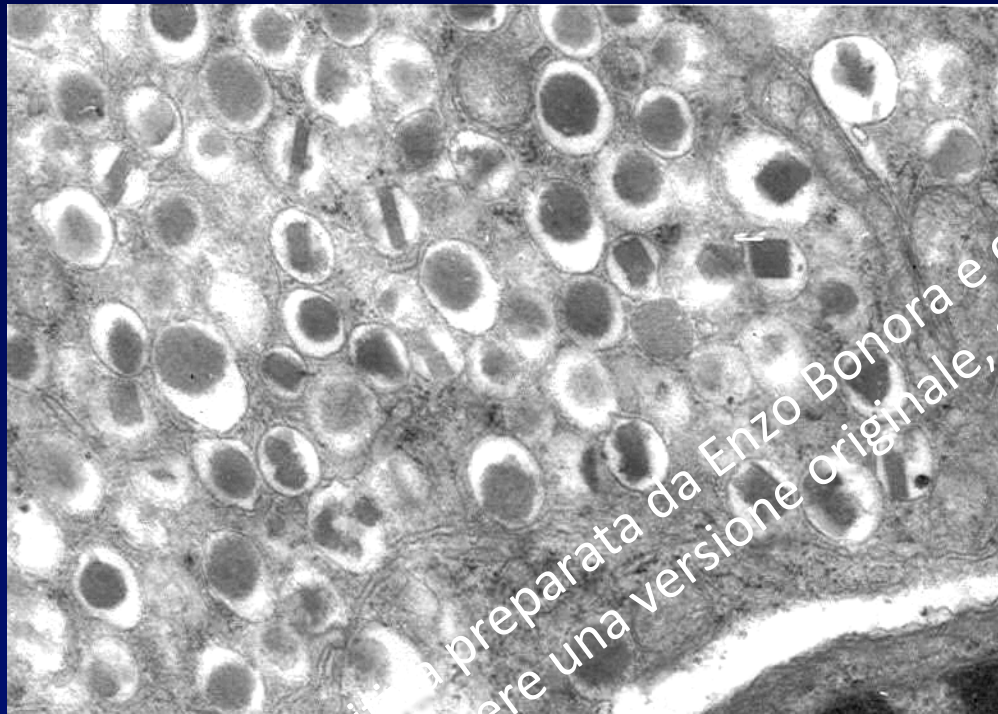
Guardado-Mendoza R et al
PNAS 2009; 106: 13992-13997

Marker of proliferation Ki67 only in alpha cells
Marker of apoptosis M30 only in beta-cells

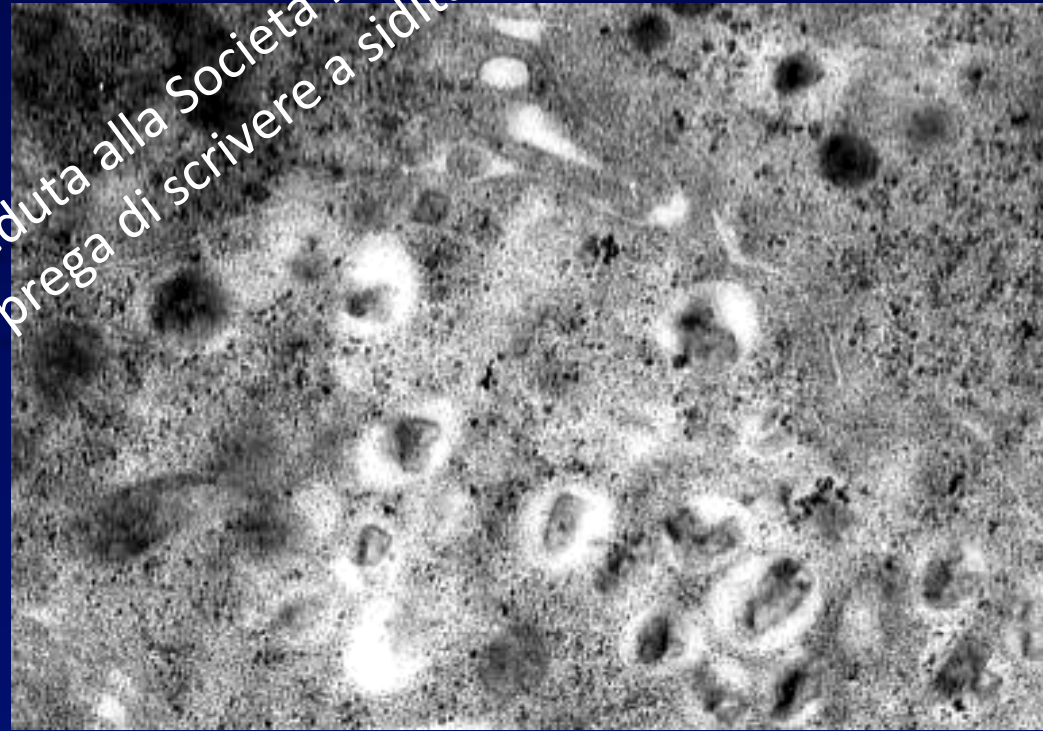


Insulin Secretory Granules Are Diminished in Human Diabetic Beta-Cells

Marchetti et al; NMCD 2006



Control

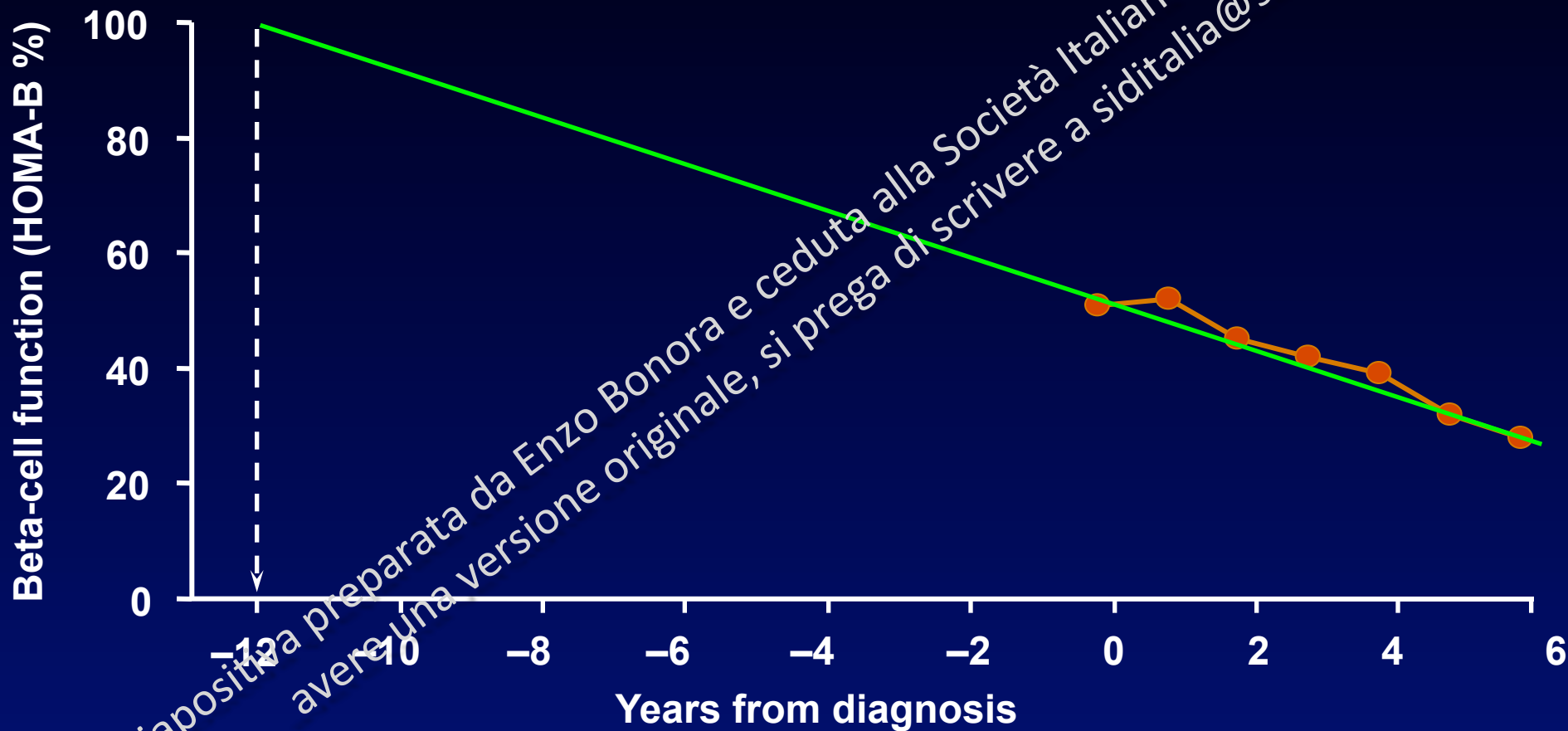


T2DM

Diapositiva preparata da Enzo Bonora e ceduta alla Società Italiana di Diabetologia. Per avere una versione originale, si prega di scrivere a siditalia@siditalia.it

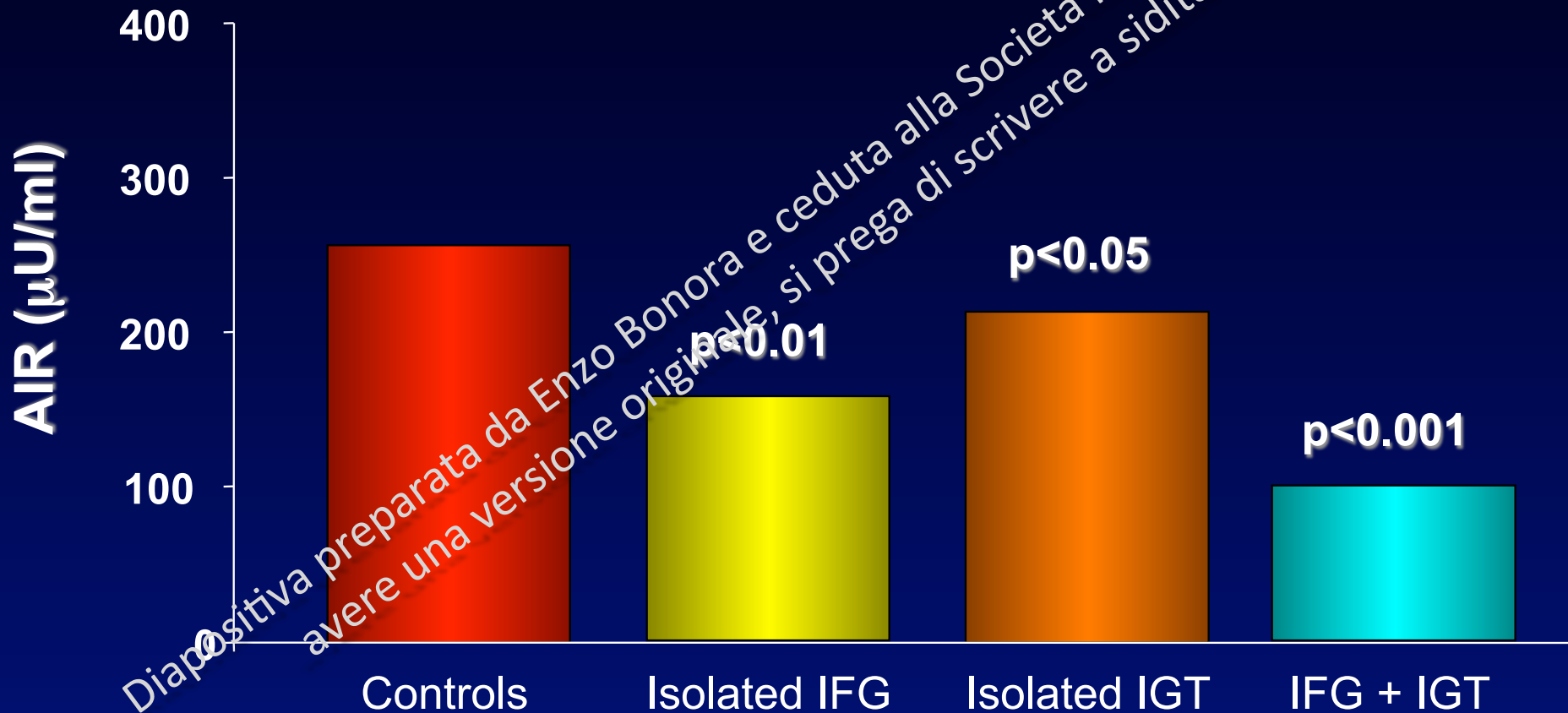
The Progression of Beta-Cell Dysfunction in T2DM

UKPDS 16 - Diabetes 1995; 44: 1249



Insulin Response to Intravenous Glucose According to Glucose Tolerance in 434 Pima Indians

Weyer et al - Diabetes 1999; 48: 2197



First- and Second-Phase Beta-Cell Secretion during Hyperglycemic Clamp and Beta-Cell Performance after OGTT in Normoglycemic Offspring of T2DM

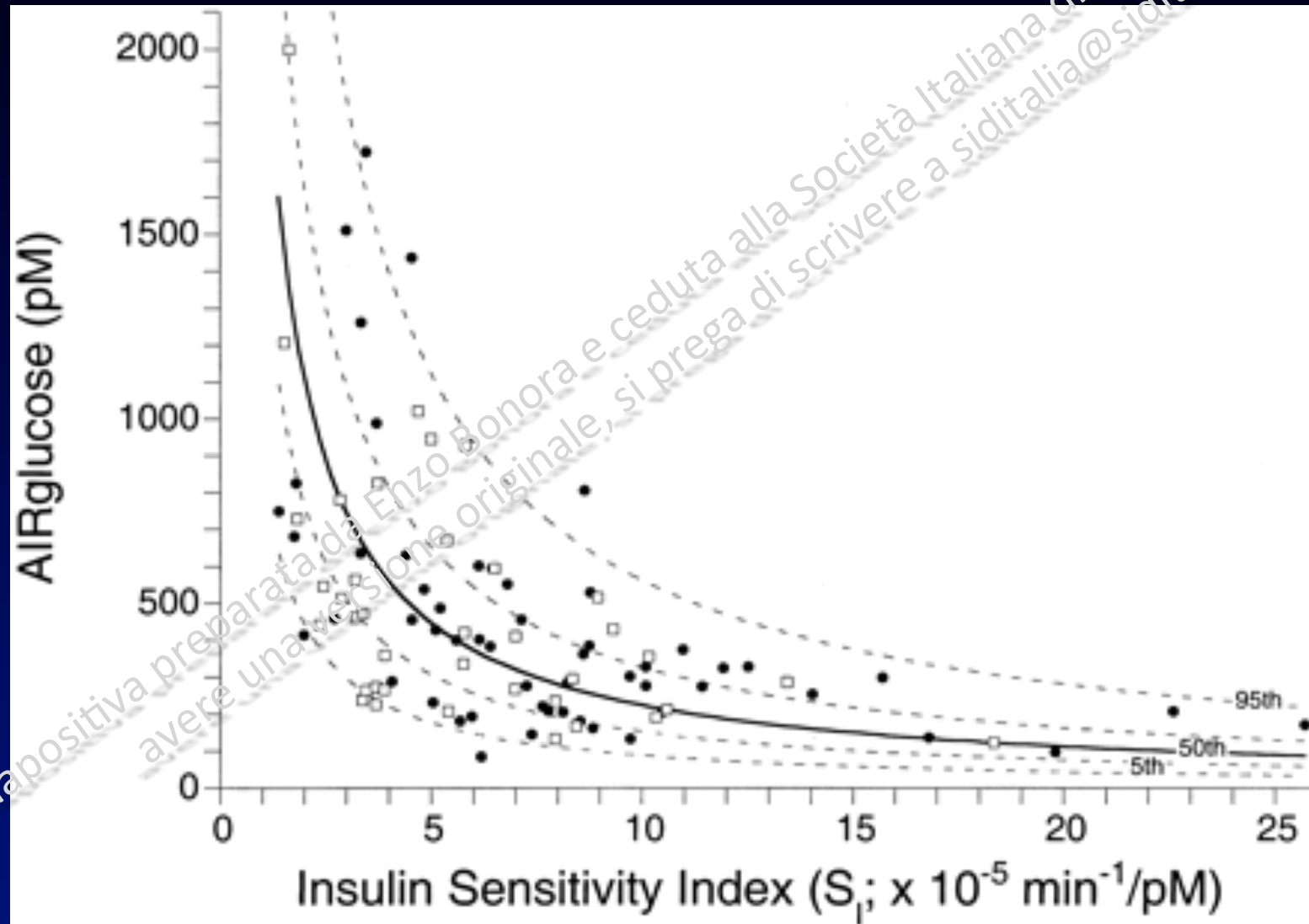
Bonadonna et al - Diabetes 2003; 52: 470

	Controls	OfT2DM	p
FPG (mmol/l)	4.5	4.6	NS
2h-PG (mmol/l)	4.5	4.8	NS
1st Phase Secretion (pmol/m ² BSA)	4459	3174	0.01
2nd Phase Secretion (pmol/m ² BSA)	68334	50286	0.02
OGTT β -index (log pmol/min.m ² BSA)	5.6	5.1	0.01

N=38, Age 46, BMI 26.5

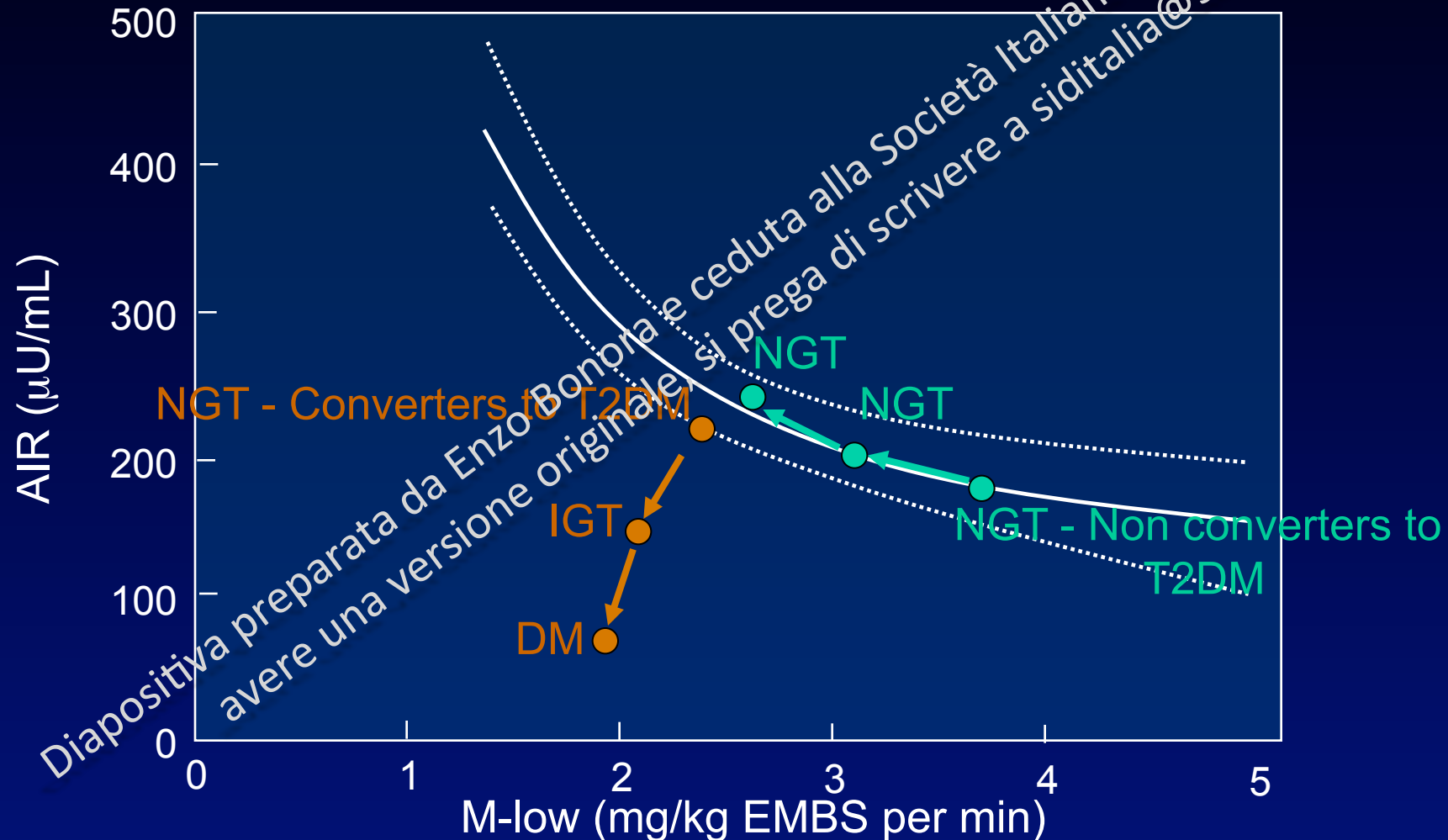
Relationship of First-Phase Insulin Secretion after i.v. Glucose and Insulin Sensitivity in Healthy Subjects

Kahn et al - Diabetes 1993; 42: 1663



Changes in Insulin Secretion and Sensitivity are Associated to the Development of T2DM

Weyer C et al - J Clin Invest 1999; 104:787



Indipendent Predictors of HbA1c in Newly Diagnosed Type 2 Diabetic Subjects

Bonora and Bonadonna - unpublished data

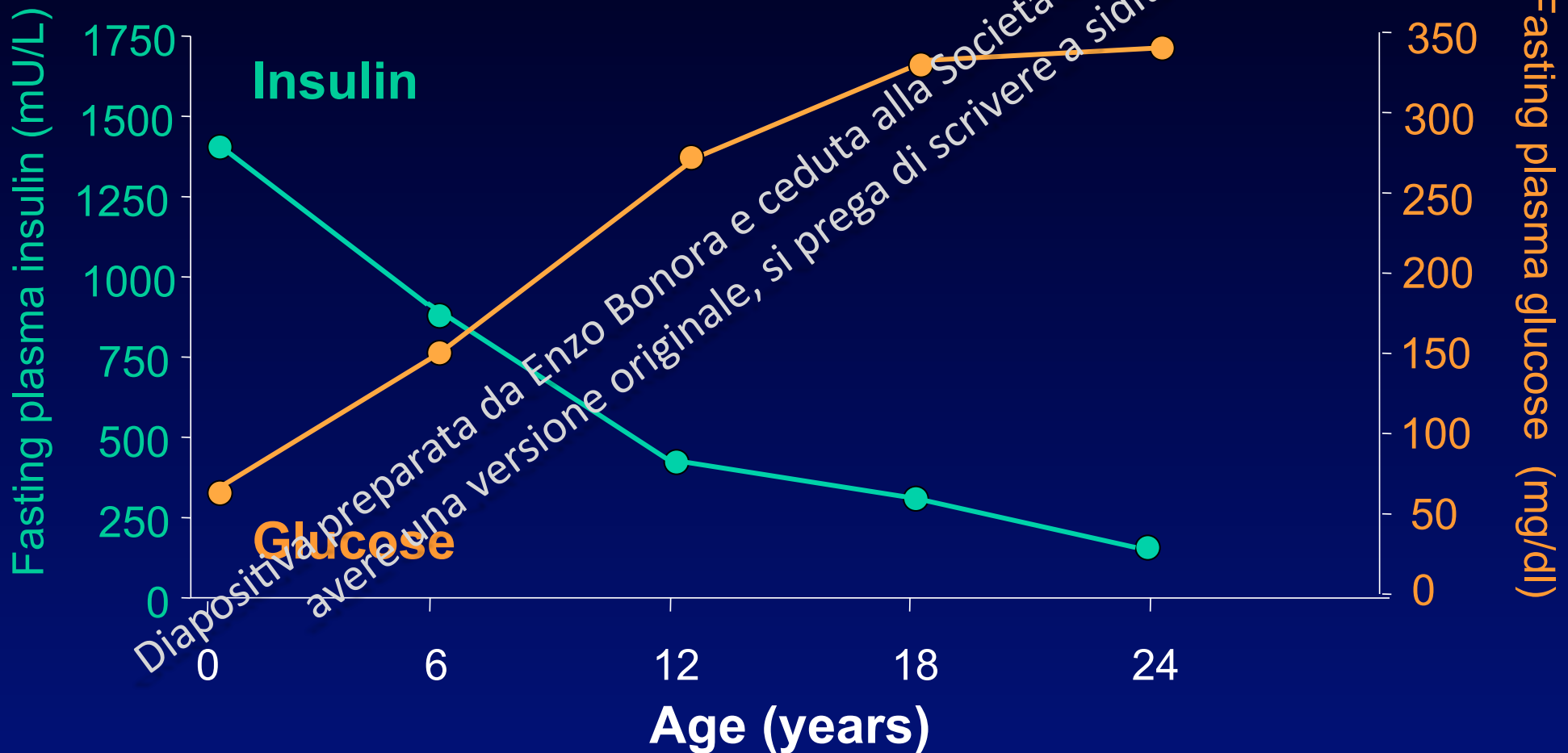
Independent variables	Standardized β -Coefficient	p
Age	0.234	0.001
β -index	- 0.386	0.001
M-clamp	-0.177	0.001

Explained variance: 24.6%

Studi in modelli animali e umani

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A Proof-of-Concept from Mother Nature: A Girl with Leprechaunism



The main underlying disorders in T2DM

Insulin Resistance

- not sufficient even when extremely severe
- perhaps not necessary, if beta-cell dysfunction is very severe

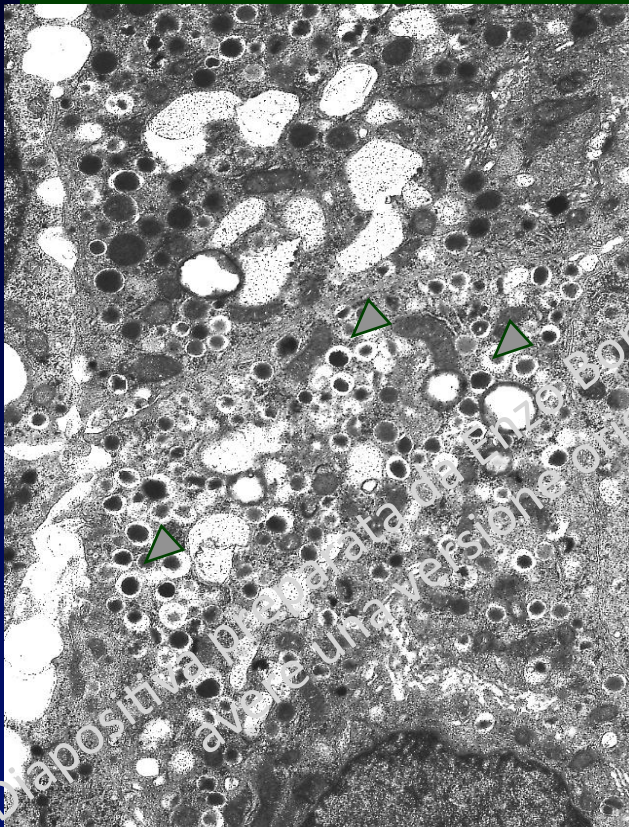
Beta-Cell Dysfunction

- absolutely necessary
- probably sufficient, if severe enough

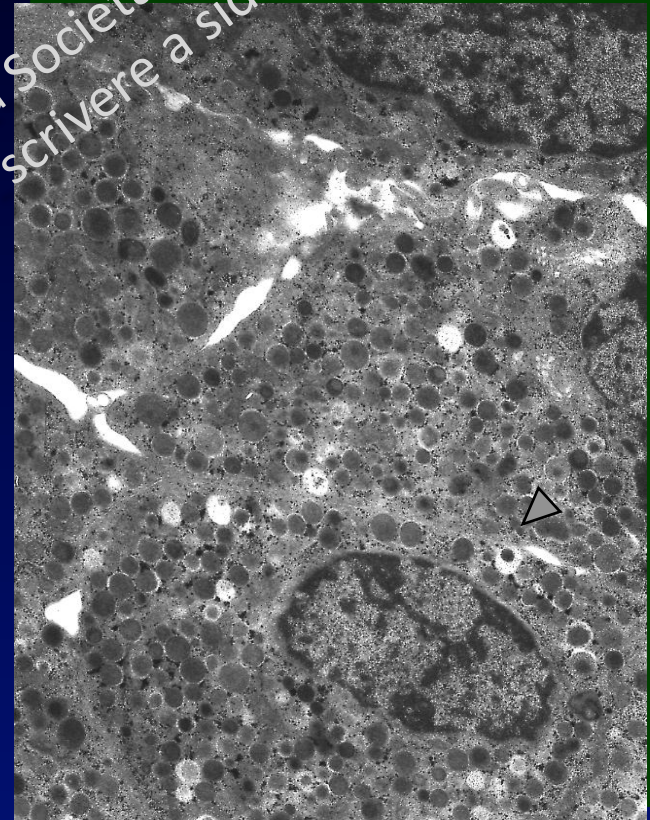
IRS-1 genetic variant reduces mature insulin granules in human islets

Marchetti P et al - Diabetes 2002; 51:1419

Gly/Gly

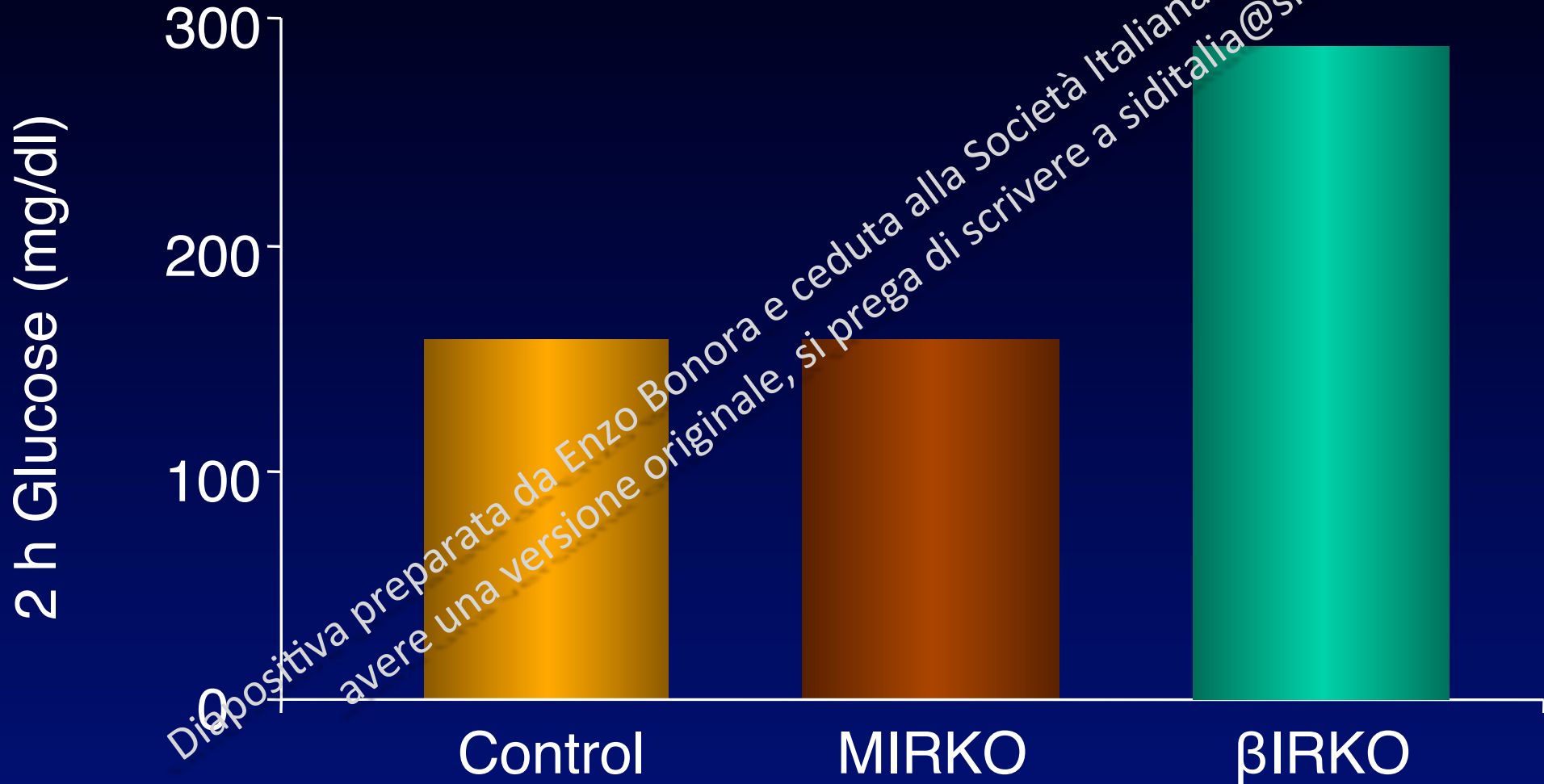


Arg



Transgenic mice and glucose regulation

Mauvais-Jarvis et al - Diabetes 2000; 49: 2126



Conclusions

- With a dynamic, rather than static, assessment of parameters, beta-cell dysfunction clearly appears to contribute to T2DM development more strongly than insulin resistance.
- T2DM cannot develop without beta-cell dysfunction.
- Virtually all subjects with T2DM have beta-cell dysfunction.
- In T2DM beta-cell dysfunction contributes to hyperglycemia more than insulin resistance.
- Beta-cell dysfunction and reduced beta-cell mass can be found many years before diabetes is diagnosed and they progressively worsen over the years dictating the loss of metabolic control.
- However, insulin resistance and beta-cell dysfunction are intimately associated and insulin resistance is an important promoting effect.

Fine

Grazie per l'attenzione

Diapositiva preparata da Enzo Bonora e ceduta alla Società Italiana di Diabetologia. Per avere una versione originale, si prega di scrivere a siditalia@siditalia.it