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Evaluation of genetic predisposition to insulin resistance by nutrient-induced insulin output ratio (NIOR)[1)]

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Abstract

Background: New tools to identify genotype-phenotype interactions need to be described and implemented. The aim of this study was to identify correlation between the risk originating from gene variation and diet-dependent development of insulin resistance.
Methods: Insulin output in terms of area under the curve after an oral glucose tolerance test (AUC Ins OGTT) and lipid tolerance tests (AUC Ins OLTT) were measured in 167 overweight/obese patients. Estimation of the 18 common gene polymorphisms for obesity risk and standard phenotyping were performed.
Results: Insulin output (AUC Ins OGTT) correlated strongly between both insulin treatments across the whole group. However, within the genotype subgroups, correlation was lower or did not exist. Using a nutrient-induced insulin output ratio (NIOR), calculated as AUC Ins OLTT/AUC Ins OGTT, values ranged from 0.42 to 5.83 and correlated significantly with body mass index (BMI) and leptin, but not with age, gender, waist-to-hip ratio (WHR) and homeostasis model assessment of insulin resistance (HOMA-IR) or plasma adiponectin. High NIOR was found in a subgroup of carriers of rare allelic variants of genes characteristic for poorer tolerance to lipids in the diet. Low NIOR values were found within a sub-group with rare genetic variants regulating carbohydrate metabolism. Thus, the new insulin index NIOR may distinguish gene variant carriers into groups of glucose- or lipid-sensitive phenotypes.
Conclusions: We suggest that the OLTT/OGTT insulin output ratio (NIOR) may be predictive for identifying individuals who are phenotypically susceptible to insulin resistance in response to high fat or carbohydrate in their habitual diet.
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Keywords: gene polymorphisms; individualized nutrition; insulin resistance; metabolic syndrome; obesity; prognostic marker.

Introduction

Metabolic syndrome and obesity are now considered to be chronic, genome-based diseases, the etiology of which may be influenced at many stages by nutritional and metabolic factors (1). The obesity phenotype is the result of the interaction of both genetic and environmental influences (2). The strongest evidence for an environment effect is that for diet. It is estimated that up to 50% of the obesity phenotype and insulin resistance may be controlled by diet (i.e., bioactive dietary compounds including carbohydrates, amino acids, fatty acids and structural lipids, minerals, and vitamins) as well as associated lifestyle factors (3).

Current dietary guidelines are based mainly on epidemiological data (4). However, the response to diet differs in many ways because of inter-individual variations in genetic, epigenetic, and metabolic phenotypes (5, 6) Thus, to transform current population-based dietary guidelines into personalized genetic-polymorphism-related dietary recommendations, healthcare experts need tools to investigate and characterize phenotype-genotype interactions. Such tools will provide insight into metabolic status based on nutrient-specific responses, as well as genotype, determining ‘‘metabolic phenotype’’ and dietary susceptibility to disease (5, 6).

Metabolic regulation, from genes to metabolites, dictates biochemical functions, as well as the nutritional and dietary needs of an individual. Therefore, the description of genetic disposition and metabolic needs are important for determining the optimal diet for any individual (7). Assessment of the long-term risk of disease and personalized dietary recommendations can be made based on an individual’s genotype and metabolic phenotype, which is derived from the complex interaction of the genotype-phenotype continuum in the context of an individual’s life stage, lifestyle, and environment (8). Unfortunately, medical experts and dietitians do not yet have such instruments to evaluate the risks or benefits derived from the individual genotype or proposed dietary regimen (6).

Early recognition and detection of dietary glucose- or fat-induced insulin resistance would be beneficial not only for reducing disease impact (9), but also for identification of those who will respond to a particular diet and, importantly, those who will not (10, 11).

In this article we propose a new way of evaluating the risk of insulin resistance in a group of people car-

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rying alleles that predispose individuals to obesity and related complications. A new insulin index, the nutrient-induced insulin output ratio (NIOR), is based on assessment of insulin release after a standard oral glucose tolerance test or high-fat meal.

Materials and methods

Patients

Families were selected from patients referred to the outpatient clinic of the lipid disorders and obesity unit at the Department of Clinical Biochemistry, Jagiellonian University, Medical College, Krakow. The study protocol was carried out under the guidance and approval of the Local Ethical Committee (no. KBET 250/B/2002). Volunteers were included only after providing written informed consent.

Patients were asked to maintain their usual diet, not to use drugs affecting lipid or glucose metabolism for at least 2 weeks, and to refrain from smoking, consuming alcohol and caffeine for at least 3 days prior to the start of the study, when blood pressure, body mass index (BMI) and waist-to-hip ratio (WHR) were measured.

The whole study group consisted of 167 patients (107 women and 60 men). The average (mean±SD) age of female and male patients was 44.1±16.3 and 39.6±17.1 years, respectively. Collectively, they represented 94 obese families (i.e., BMI>30 kg/m^2 measured in at least one member of the family). The distribution of lean and overweight subjects (BMI<30 kg/m^2, group 1) and obese (BMI≥30 kg/m^2, group2) in the study group was 29.9% and 70.1%, respectively. Individuals with significant comorbidities or undergoing lipid-lowering therapy were excluded from the study.

Biochemical analyses

Biochemical tests were performed after overnight fasting (12 h). Plasma total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), glucose, insulin (immunoradiometric assay, IRMA; ####, Świerk, Poland), and leptin (IRMA, Linco Research Inc., ####, USA) and adiponectin (ELISA, R&D Systems, ####, USA) were measured using routine biochemical methods. The inter- and intra-assay variability was below 5% in all cases.

A standard oral glucose tolerance test (OGTT; after 75 g of glucose) and oral lipid tolerance test (OLTT) after a high-fat meal [1033-kcal breakfast composed of 40% carbohydrates, 20% protein and 40% fat (50% saturated, 40% monounsaturated and 10% polyunsaturated fatty acids) according to Couderc et al. (12)] were performed.

Insulin levels were evaluated every 30 min up to 2 h following oral glucose challenge and every 2 h up to 8 h following a high-fat meal. The results are expressed as area under the curve for insulin (AUC Ins), which was calculated using trapezoidal integration. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated according to the formula [insulin (μU/mL)×glucose (mmol/L)]/22.5 (13). The nutrient-induced insulin output ratio (NIOR) was calculated as AUC Ins OLTT/AUC Ins OGTT. The relationship between specific alleles and NIOR was expressed as the percentage relative difference between opposite allele carriers, calculated according to relative difference=[{NIOR BB (or NIOR AB)–NIOR AA}/NIOR AA]×100, where AA indicates the common (reference sequence) genotype; BB indicates the rare genotype (variant) and AB indicates the heterozygous genotype.

This comparison of relative differences between different allele carriers allows the extent of changes between allele carriers to be determined (14).

Genotyping

Genomic DNA was extracted from cellular blood components using a QIAamp® Blood Kit (Qiagen, Inc., ###). PCR amplification of the gene fragments from genomic DNA was performed using HotStar polymerase from Qiagen and specific primers. The mutations examined are listed in Table 1 according to their location in the human genome.

Genotyping of peroxisome proliferator-activated receptor-γ2 (*PPAR-γ2*), cholesteryl ester transfer protein (*CETP*), *Hin*dIII (*LPL-H*) and *Pvu*II (*LPL-P*) polymorphisms of the lipoprotein lipase gene, apolipoprotein CIII (*apoCIII*), uncoupling protein-1 (*UCP-1*), β_2-adrenergic receptor (*b2AR*), β_3-adrenergic receptor (*b3AR*), dopaminergic D2 receptor (*DRD2*), tumor necrosis factor-α (*TNF-α*), *HSP-70-2*, *HSP-70hom*, *FoxC2*, *SRBI*, and *CFR13I* (CD36/FAT) genes was performed using the restriction fragment length polymorphism (RFLP) method, which is described elsewhere (15–30). PCR conditions were matched to the melting points of specific primers. The restriction enzymes were characteristic for the site of

Table 1 List of the mutations studied.

Gene	Mutation	Amino acid	Location	Chromosome	Reference
PPAR-γ2	C→G	Pro→Ala	Codon 12	3p25	(15)
CETP	G→A	–	Intron 1	16q21	(16)
LPL-H	T→G	–	Intron 8	8p22	(17)
LPL-P	C→T	–	Intron 6	8p22	(17)
apoCIII	C→G	–	3′ non-coding sequence	11q23	(18)
UCP-1	A→G	–	–3826 (promoter)	4q31	(19)
b2AR	C→G	Gln→Glu	Codon 27	5q32–q34	(20)
b3AR	T→G	Trp→Arg	Codon 64	8p12–p11.2	(21)
DRD2	T→C		3′ non-coding sequence	11q23	(22)
TNF-α	G→A		–308 (promoter)	6p21.3	(23)
HSP-70-2	A→G	Silent (Gln)	Codon 352	14q24.1	(24)
HSP-70hom	T→C	Met→Thr	Codon 493	5q31.1–q31.2	(24)
FoxC2	C→T	–	–512 (promoter)	16q24.3	(25)
FABP-1	A→G	–	Intron 8	2p11	(26)
MCR-3	G→A	Ile→Val	Codon 81	20q13.2	(27)
SRBI	C→T	Silent (Arg)	Codon 419	12q24.31	(28)
CFR13I	C→T	Pro→Ser	Codon 90	7q11.2	(29)
apoE 2/3, 3/3, 4/3	ε2 (112Cys158Cys)	ε3 (112Cys158Arg)	ε4 (112Arg158Arg)	19q13.32	(30)

mutation. Genotyping was based on the variation of band pattern (product length) detected in gel electrophoresis. The common polymorphism of *apoE* (30) was detected using the hybridization method with a commercially available kit (Pharmacia Biotech, ####). Fatty acid transport protein 1 (*FATP-1*) (polymorphism A>G in intron 8) (26) and melanocortin receptor-3 (*MCR-3*) (polymorphism A>G in codon 81 Ile>Val) (27) genotyping was performed by direct sequencing using a Visible Genetics automated sequencer (####) according to the manufacturer's instructions.

Statistical methods

Descriptive results for continuous variables are expressed as mean±SD. Normal distribution and homogeneity of variables were tested before statistical analysis was undertaken. Differences between variables in the genotype groups were compared by analysis of variance (ANOVA). The level of statistical significance was set at $p<0.05$. Statistical analysis was performed using Statistica 7.0 for Windows from Statsoft (####).

Results

Descriptive characteristics of the study group are shown in Table 2. High BMI index was associated with dyslipidemia (i.e., increased levels of serum TG, free fatty acids and serum cholesterol) and insulin resistance, as determined by elevated insulin and HOMA-IR.

The differences in insulin secretory response after a glucose or lipid load between lean and overweight individuals (group 1, BMI<30 kg/m^2) and obese patients (group 2, BMI≥30 kg/m^2) are presented in Table 3. Those with a lower BMI demonstrated higher insulin sensitivity (i.e., lower fasting plasma glucose, insulin levels, and AUC Ins during OGTT and OLTT). Lower BMI was also associated with better glucose- and lipid-induced insulin resistance parameters (Table 3). Elevated NIOR in obese individuals (statistically significant for the whole group, as well as for females alone) is evident in Table 3.

Strong correlation between insulin output (AUC Ins) during OGTT and OLTT (Table 4), which was not dependent on age or gender, was also observed.

The distribution of different genotypes within the study group is shown in Table 5. The relationship between common polymorphisms reported to be associated with obesity or its complications and their effect on plasma insulin output (AUC Ins) during OGTT and OLTT challenge is shown in Figure 1. It is evident that genetic traits may contribute to individual response to diet (Figure 1).

To visualize the differences in glucose and lipid responses between the genotype sub-groups, a new insulin index – based on individual AUC Ins during OLTT and OGTT – was developed. The mean value of the NIOR index for the whole group was 1.66±0.71, which was not influenced by gender, and ranged from

Table 2 Biochemical characteristics of participants with BMI<30 kg/m^2 (group 1) and BMI≥30 kg/m^2 (group 2) for the whole group and gender sub-groups.

	Group 1 (n=50)		Group 2 (n=117)		p
	Mean	SD	Mean	SD	
Total group (n=167)					
BMI, kg/m^2	25.67	2.73	36.94	5.34	<0.0001
WHR	0.83	0.07	0.92	0.10	<0.0001
Leptin, ng/mL	12.27	11.64	29.70	15.34	<0.0002
Adiponectin, μg/mL	9.50	5.10	7.36	3.85	<0.0001
Total cholesterol, mmol/L	4.56	0.94	5.62	1.76	<0.0001
HDL-cholesterol, mmol/L	1.40	0.29	1.35	0.29	n.s.
LDL-cholesterol, mmol/L	2.59	0.98	3.23	1.04	<0.0001
Triglycerides, mmol/L	1.34	1.00	2.28	3.68	<0.05
HOMA-IR	2.17	1.57	4.39	3.41	<0.0001
Females (n=107)	Group 1 (n=32)		Group 2 (n=75)		
BMI, kg/m^2	25.66	2.78	36.44	5.03	<0.0001
WHR	0.79	0.05	0.87	0.06	<0.0001
Leptin, ng/mL	16.45	12.87	34.72	14.82	<0.0001
Adiponectin, μg/mL	11.36	5.70	9.82	3.63	<0.005
Total cholesterol, mmol/L	4.55	0.88	5.62	1.22	<0.0001
HDL-cholesterol, mmol/L	1.46	0.33	1.45	0.28	n.s.
LDL-cholesterol, mmol/L	2.57	0.94	3.37	1.12	<0.0001
Triglycerides, mmol/L	1.16	0.96	1.67	0.83	<0.05
HOMA-IR	2.41	1.85	3.96	3.47	<0.05
Males (n=60)	Group 1 (n=18)		Group 2 (n=42)		
BMI, kg/m^2	25.69	2.72	37.80	5.80	<0.0001
WHR	0.88	0.05	1.02	0.07	<0.0001
Leptin, ng/mL	5.30	3.05	20.42	11.62	<0.0001
Adiponectin, μg/mL	7.06	2.86	4.64	1.62	<0.0005
Total cholesterol, mmol/L	4.58	1.07	5.63	2.43	<0.01
HDL-cholesterol, mmol/L	1.30	0.15	1.17	0.23	<0.05
LDL-cholesterol, mmol/L	2.62	1.08	2.98	0.86	n.s.
Triglycerides, mmol/L	1.66	1.01	3.33	5.85	n.s.
HOMA-IR	1.75	0.79	5.11	3.22	<0.0001

Table 3 Mean insulin output during OGTT and OLTT in participants with BMI<30 kg/m² (group 1) and BMI≥30 kg/m² (group 2) in the whole group and gender sub-groups.

	Group 1 (n=50)		Group 2 (n=117)		p
	Mean	SD	Mean	SD	
Whole group (n=167)					
OGTT					
Insulin 0 min	10.34	6.66	16.83	10.79	<0.0001
Insulin 30 min	78.60	57.85	107.94	68.82	<0.005
Insulin 60 min	86.74	62.29	144.18	97.23	<0.0001
Insulin 90 min	64.25	57.36	129.07	100.28	<0.0001
Insulin 120 min	43.21	51.04	90.65	92.33	<0.0002
AUC Ins OGTT	7208.4	1131.970	13247.8	735.705	<0.0001
OLTT					
Insulin 0 h	9.51	7.95	17.10	10.59	<0.0001
Insulin 2 h	44.31	46.72	102.76	91.34	<0.0001
Insulin 4 h	26.64	49.80	40.40	56.68	n.s.
Insulin 6 h	10.00	11.17	17.44	15.46	<0.005
Insulin 8 h	7.70	5.96	12.53	9.12	<0.0001
AUC Ins OLTT	10214.8	1976.8	21434.1	1284.8	<0.0001
NIOR	1.45	0.66	1.75	0.82	0.0218
Women (n=107)	Group 1 (n=32)		Group 2 (n=75)		
OGTT					
Insulin 0 min	11.85	7.80	15.45	10.83	n.s.
Insulin 30 min	89.35	66.69	102.43	73.29	n.s.
Insulin 60 min	95.50	70.13	128.44	95.58	n.s.
Insulin 90 min	72.46	71.75	120.38	103.52	<0.02
Insulin 120 min	53.55	65.19	88.39	93.43	<0.05
AUC Ins OGTT	8075.2	1535.4	12385.9	993.816	<0.02
OLTT					
Insulin 0 h	10.29	9.44	15.39	9.67	<0.005
Insulin 2 h	50.70	57.06	94.62	86.41	<0.003
Insulin 4 h	34.84	61.59	36.84	38.78	n.s.
Insulin 6 h	11.19	13.23	16.82	16.37	n.s.
Insulin 8 h	8.38	6.66	12.03	9.82	<0.05
AUC Ins OLTT	11871.4	2576.0	19895.9	1667.3	<0.01
NIOR	1.41	0.55	1.82	0.88	0.0203
Men (n=60)	Group 1 (n=18)		Group 2 (n=42)		
OGTT					
Insulin 0 min	8.41	7.80	15.45	10.83	n.s.
Insulin 30 min	64.78	66.69	102.43	73.29	n.s.
Insulin 60 min	75.17	70.13	128.44	95.58	n.s.
Insulin 90 min	53.70	71.75	120.38	103.52	<0.02
Insulin 120 min	29.55	65.19	88.39	93.43	<0.05
AUC Ins OGTT	5715.7	1544.6	14766.4	1011.2	<0.0001
OLTT					
Insulin 0 h	8.22	9.44	15.39	9.67	<0.005
Insulin 2 h	33.89	57.06	94.62	86.41	<0.003
Insulin 4 h	12.96	61.59	36.84	38.78	n.s.
Insulin 6 h	7.93	13.23	16.82	16.37	n.s.
Insulin 8 h	6.55	6.66	12.03	9.82	<0.05
AUC Ins OLTT	7361.6	2985.9	24144.2	1954.7	<0.0001
NIOR	1.51	0.83	1.64	0.69	n.s.

a minimum of 0.42 to a maximum value of 5.83. The NIOR distribution shows a left-skew-shaped curve, i.e., the data are not normally distributed. However, the mean and median values for men and women do not differ significantly. The median value for all participants in the study was 1.45 (1.51 for women and 1.42 for men). Regression analysis showed a significant correlation for NIOR with BMI and leptin, but not age, WHR, HOMA-IR or adiponectin levels (Table 6).

Higher NIOR values pointed to increased insulin output after a lipid-containing meal. Conversely, lower NIOR values indicated higher insulin output after glucose challenge. This observation allowed us to rank the genotype sub-groups according to their NIOR value (Table 7), segregating the different genotype carriers into responders to fat- or carbohydrate-sus-

Table 4 Correlation coefficients between insulin output after OGTT and OLTT.

	AUC correlation between Ins OGTT and Ins OLTT	
	r	p
Whole group (n=167)	0.819	<0.00001
Females (n=107)	0.822	<0.00001
Males (n=60)	0.816	<0.00001

Table 5 Genotype frequency of the polymorphisms studied for 167 patients who completed the OGTT and OLTT.

Gene	Genotype frequency, %		
PPAR-γ2 C→G	CC	CG	GG
	63.6	32.5	3.9
CETP G→A	GG	GA	AA
	26.4	45.9	27.7
LPL-H T→G	TT	TG	GG
	53.4	37.1	9.5
LPL-P C→T	CC	CT	TT
	25.7	47.3	27.0
apoCIII G→C	GG	GC	CC
	80.2	19.1	0.7
UCP-1 A→G	AA	AG	GG
	44.0	44.0	12.0
b2AR C→G	CC	CG	GG
	37.1	42.8	20.1
b3AR T→G	TT	TG	GG
	81.9	17.5	0.6
DRD2 C→T	CC	CT	TT
	60.8	37.2	2.0
TNF-α G→A	GG	GA	AA
	66.7	31.4	1.9
HSP-70-2 G→A	GG	GA	AA
	38.0	44.7	17.3
HSP-70hom T→C	TT	TC	CC
	66.2	24.8	9.0
FoxC2 C→T	CC	CT	TT
	24.8	58.2	17.0
FABP-1 A→G	AA	AG	GG
	41.2	47.0	11.8
MCR-3 G→A	GG	GA	AA
	89.8	8.2	2.0
SRBI C→T	CC	CT	TT
	28.4	45.4	26.2
CFR13I/CD36 C→T	CC	CT	TT
	91.7	4.8	3.5
apoE 2/3, 3/3, 4/3	2/3	3/3	4/3
	9.2	78.7	12.1

ceptible phenotypes (Figure 2). Screening the genotypes according to this index showed that the highest NIOR value was found for *LPL-P* TT carriers, whilst the lowest NIOR value occurred in *MCR3* GA carriers. The *MCR-3* G→A, *FABP-1* A→G, *b3AR* T→G, *TNF-α* G→A, *HSP-70-2* G→A, *apoCIII* G→C and *HSP-70hom* T→C rare allele carriers were typically found in the glucose-susceptible phenotype. In comparison, the rare allelic sub-groups of *apoE* 4/3 and 2/3, *LPL-H* T→G, *FoxC2* C→T, *UCP-1* A→G, *PPAR-γ2* C→G, *b2AR* C→G, *DRD2* C→T, *CFR13I/CD36* C→T and *LPL-P* C→T were found in the fat-susceptible phenotype (Figure 2).

Discussion

OGTT has traditionally been used to classify the status of glucose tolerance for diagnostic purposes in terms of normal (NGT) vs. impaired glucose tolerance (IGT) or diabetic (31, 32). Fat-loading tests have been used for evaluation of post-prandial hypertriglyceridemia and lipase insufficiency (33–35). Several post-prandial tests have been developed to assess post-prandial lipemia, but parallel insulin or insulin-sensitivity evaluation has not used as frequently.

The recent paper of Harano et al. (36), as well as the present study, points to a strong correlation between insulin output after glucose and standard high-fat diet tests. Insulin plays a predominant role in adipogenesis; the net effect of insulin is to enhance metabolic substrate storage and inhibit free fatty acid mobilization (37). Indeed, high levels of circulating fatty acids are responsible for hyperinsulinemia, and uptake and conversion of glucose to triglycerides or glycogen is regulated by insulin (37). Chronic high concentrations of insulin lead to further impairment of tissue insulin sensitivity and other abnormal metabolic consequences (37).

To date, only a few studies have evaluated genetic variability in aspects of response to diet. Recently, it was shown that carriers of the hepatic HDL receptor (*SRBI*) mutation are sensitive to different fatty acids

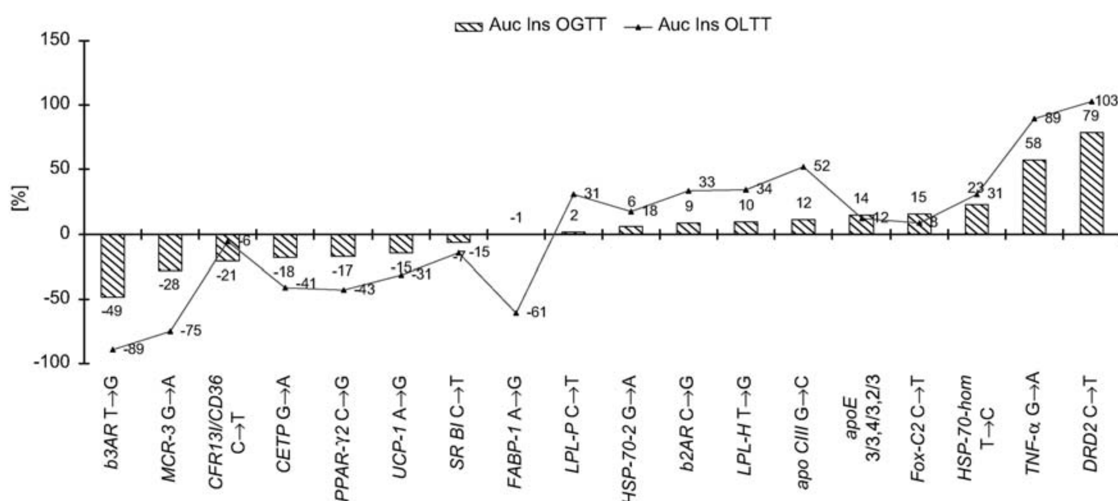

Figure 1 Ranking of the ''candidate genes'' according to AUC Ins during OGTT. The ranking is different for OLTT. Relative differences expressed as the individual percentage AUC Ins determined by certain alleles according to the formula [{(AUC Ins (BB)–AUC Ins (AA)}/AUC Ins (AA)]×100.

Table 6 Correlation coefficients between NIOR and other variables in the whole group and gender sub-groups.

	Whole group		Females		Males	
	r	p	r	p	r	p
BMI	**0.2503**	**0.0011**	**0.2335**	**0.0165**	**0.2965**	**0.0214**
WHR	0.0009	0.9917	0.0241	0.8218	0.2172	0.1297
Adiponectin	–0.0690	0.5701	–0.0704	0.6743	–0.0628	0.7324
Total cholesterol	0.0382	0.6271	**0.2267**	**0.0206**	–0.1391	0.2891
HDL-cholesterol	–0.0671	0.3931	–0.0679	0.4928	–0.1488	0.2562
LDL-cholesterol	0.1232	0.1170	**0.2498**	**0.0105**	–0.1469	0.2665
Fasting glucose	0.1167	0.1353	0.0229	0.8160	**0.2797**	**0.0304**
HOMA-IR	0.0808	0.3016	–0.0471	0.6326	**0.3065**	**0.0164**
Triglycerides	–0.0469	0.5506	0.0396	0.6896	–0.0787	0.5499
Leptin	**0.2676**	**0.0005**	**0.2928**	**0.0023**	0.2071	0.1187

Significant correlations for NIOR with BMI and leptin (in bold) were observed.

in the diet compared to those with the reference sequence. The authors demonstrated that carriers of the G/A genotype have significantly increased insulin sensitivity after a monounsaturated fatty acid-rich diet compared to G/G individuals (38). Other authors demonstrated that patients homozygous for the –455C *APOC3* variant are poorly responsive to the *apoCIII*-lowering effects of n-3 polyunsaturated fatty

Table 7 NIOR values according to genotype sub-groups and relative differences between homozygotes (occurring frequently) and heterozygotes or rare homozygotes.

Gene	NIOR			SD			Relative difference, %	
							Heterozygotes	Homozygotes
PPAR-γ2 C→G	CC	CG	GG	CC	CG	GG		
	1.655	1.678	1.808	0.777	0.861	1.053	1.42	9.25
CETP G→A	GG	GA	AA	GG	GA	AA		
	1.668	1.661	1.630	0.754	0.826	0.720	–0.46	–2.32
LPL-H T→G	TT	TG	GG	TT	TG	GG		
	1.496	1.738	1.581	0.716	0.885	0.335	16.16	5.68
LPL-P C→T	CC	CT	TT	CC	CT	TT		
	1.515	1.727	1.809	0.688	0.881	0.781	14.02	19.44
apoCIII G→C	GG	GC	CC	GG	GC	CC		
	1.661	1.648	1.540	0.728	1.046		–0.77	–7.28
UCP-1 A→G	AA	AG	GG	AA	AG	GG		
	1.563	1.761	1.682	0.695	0.885	0.799	12.72	7.66
b2AR C→G	CC	CG	GG	CC	CG	GG		
	1.654	1.606	1.820	0.857	0.704	0.865	–2.90	10.05
b3AR T→G	TT	TG	GG	TT	TG	GG		
	1.714	1.410	1.421	0.831	0.583		–17.72	–17.11
DRD2 C→T	CC	CT	TT	CC	CT	TT		
	1.554	1.812	1.729	0.808	0.787	1.311	16.57	11.25
TNF-α G→A	GG	GA	AA	GG	GA	AA		
	1.733	1.549	1.473	0.875	0.670	0.333	–10.64	–15.02
HSP-70-2 G→A	GG	GA	AA	GG	GA	AA		
	1.463	1.676	1.684	0.658	0.727	0.898	14.62	15.11
HSP-70hom T→C	TT	TC	CC	TT	TC	CC		
	1.662	1.622	1.586	0.817	0.814	0.661	–2.39	–4.55
FoxC2 C→T	CC	CT	TT	CC	CT	TT		
	1.624	1.706	1.724	0.697	0.843	0.738	5.07	6.20
FABP-1 A→G	AA	AG	GG	AA	AG	GG		
	1.749	1.943	1.063	0.784	0.978	0.354	11.09	–39.21
MCR-3 G→A	GG	GA	AA	GG	GA	AA		
	1.680	2.053	0.842	0.831	0.378		22.26	–49.85
SRBI C→T	CC	CT	TT	CC	CT	TT		
	1.624	1.654	1.657	0.807	0.646	0.950	1.88	2.04
CFR13I/CD36 C→T	CC	CT	TT	CC	CT	TT		
	1.669	1.614	1.924	0.794	0.760	0.456	–3.34	15.25
apoE 2/3, 3/3, 4/3	2/3	3/3	4/3	2/3	3/3	4/3		
	1.672	1.622	1.756	0.759	0.773	0.803	3.04*	8.26*

*Genotype E3/3 is predominant in our population.

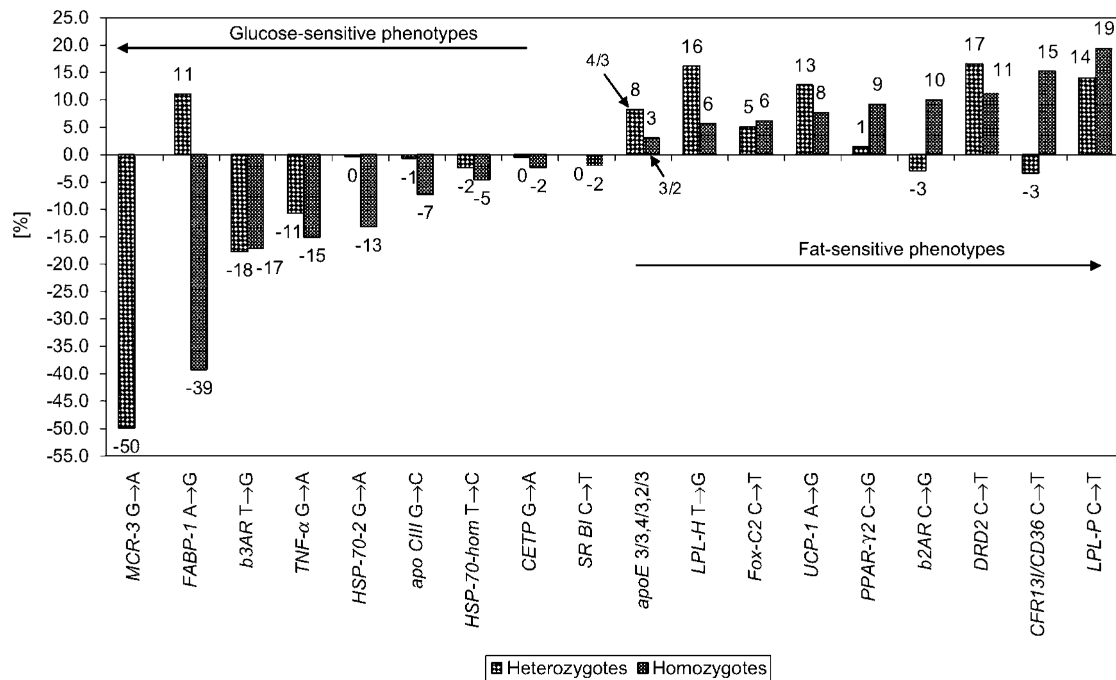

Figure 2 Ranking of genotype sub-groups according to NIOR differences between opposite allele carriers expressed as a percentage.
Relative difference = [{(NIOR (BB or AB)–NIOR (AA)}/NIOR (AA)] × 100.

acids (39). Salas et al. suggested that mutation of the *apoCIII* gene affects insulin response to carbohydrate, which results in reduced sensitivity to insulin, especially when individuals consume diets rich in saturated fat (40).

Improving nutrition is essentially a process encouraging people to make healthy choices that improve their wellbeing (41). Guiding consumers to select the most effective nutritional supplement based on their genotype is a new goal in nutritional genomics. Determination of genetic markers associated with specific diseases might allow the development of new molecular diagnostic products to indicate the risk or presence of disease before symptoms appear, predict the severity or expected rate of progression of a disease, or aid in monitoring response to therapy. The first step is to associate the occurrence of a particular genetic variant with the incidence of a particular disease or disease predisposition – an association that can vary from one individual to another. Our NIOR index could be used as such a tool to ascertain an individual's susceptibility (according to genotype) to high insulin secretion in response to a high-carbohydrate or high-lipid diet.

In this study, evidence of genotype-dependent insulin release as a result of different dietary loads is presented. The introduction of a new insulin index, NIOR, shows that it is feasible to distinguish between individuals and genetic polymorphisms according to dietary response and biochemical effects, in this case insulin output/resistance. Our study showed that *MCR-3* A, *FABP-1* G, *b3AR* G, *TNF-α* A, *HSP-70-2* A, *apoCIII* C and *HSP-70hom* C allele carriers are glucose-susceptible, while *apoE* 4/3 and 2/3, *LPL-H* G, *FoxC2* T, *UCP-1* G, *PPAR-γ2* G, *b2AR* G, *DRD2* T, *CFR13I/CD36* T and *LPL-P* T occurred in fat-susceptible phenotypes. Thus, NIOR adds new knowledge to the evaluation of early genetic markers for the detection and potentially the prevention of metabolic syndrome.

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References

1. Martin LJ, North KE, Dyer T, Blangero J, Comuzzie AG, Williams J. Phenotypic, genetic, and genome-wide structure in the metabolic syndrome. BMC Genet 2003;4 (Suppl 1):S95.
2. Desiere F. Towards a systems biology understanding of human health: Interplay between genotype, environment and nutrition. Biotechnol Annu Rev 2004;10:51–84.
3. Zeisel SH, Freake HC, Bauman DE, Bier DM, Burrin DG, German JB, et al. The nutritional phenotype in the age of metabolomics. J Nutr 2005;135:1613–6.
4. Go VL, Wong DA, Wang Y, Butrum RR, Norman HA, Wilkerson L. Diet and cancer prevention: evidence-based medicine to genomic medicine [review]. J Nutr 2004;134 (Suppl 12):3513S–6.
5. Daniel H. Genomics and proteomics: importance for the future of nutrition research [review]. Br J Nutr 2002;87 (Suppl 2):S305–11.

6. German B, Young VR. Nutrition and genomics [review]. Nestle Nutr Workshop Ser Clin Perform Programme 2004;9:243–61; discussion 261–3.
7. Go VL, Wang Y, Yang H, Lee WN. Neuro-hormonal integration of metabolism: challenges and opportunities in the postgenomic era [review]. Nestle Nutr Workshop Ser Clin Perform Programme 2004;9:227–38; discussion 238–42.
8. Muller M, Kersten S. Nutrigenomics: goals and strategies [review]. Nat Rev Genet 2003;4:315–22.
9. Harano Y, Suzuki M, Koyama Y, Kanda M, Yasuda S, Suzuki K, et al. Multifactorial insulin resistance and clinical impact in hypertension and cardiovascular diseases. J Diabetes Complications 2002;16:19–23.
10. Whitfield PD, German AJ, Noble PJ. Metabolomics: an emerging postgenomic tool for nutrition [review]. Br J Nutr 2004;92:549–55.
11. German JB, Bauman DE, Burrin DG, Failla ML, Freake HC, King JC, et al. Metabolomics in the opening decade of the 21st century: building the roads to individualized health. J Nutr 2004;134:2729–32.
12. Couderc R, Peynet J, Cambillaud M, Tallet F, Cosson C, Lefevre G, et al. Effects of postprandial hyperlipemia on the vitamin E content of lipoproteins. Clin Chim Acta 1998;277:141–52.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
14. American College of Physicians. American Society of Internal Medicine. Primer on absolute vs. relative difference. Effective Clin Pract 1999;2:46.
15. Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J. et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284–7.
16. Riemens, SC, Van Tol A, Stulp BK, Dullaart RP. Influence of insulin sensitivity and the TaqIB cholesteryl ester transfer protein gene polymorphism on plasma lecithin:cholesterol acyltransferase and lipid transfer protein activities and their response to cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), and lipoprotein lipases are involved in hyperinsulinemia in non-diabetic men. J Lipid Res 1999;40:1467–74.
17. Jemaa R, Tuzet S, Portos C, Betoulle D, Apfelbaum M, Fumeron F. Lipoprotein lipase gene polymorphisms: associations with hypertriglyceridemia and body mass index in obese people. Int J Obes Relat Metab Disord 1995;19:270–4.
18. Olivieri O, Bassi A, Stranieri C, Trabetti E, Martinelli N, Pizzolo F, et al. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. J Lipid Res 2000;44:2374–81.
19. Nagai N, Sakane N, Ueno LM, Hamada T, Moritani T. The –3826 A→G variant of the uncoupling protein-1 gene diminishes postprandial thermogenesis after a high fat meal in healthy boys. J Clin Endocrinol Metab 2003; 88:5661–7.
20. Hoffstedt J, Poirier O, Thorne A, Lonqvist F, Herrmann SM, Carnbien F, et al. Polymorphism of the human beta(3)-adrenoceptor gene forms a well-conserved haplotype that is associated with moderate obesity and altered receptor function. Diabetes 1999;48:203–5.
21. Mori Y, Kim-Motoyama H, Ito Y, Katakura T, Yasuda K, Ishiyama-Shigemoto S, et al. The Gln27Glu beta2-adrenergic receptor variant is associated with obesity due to subcutaneous fat accumulation in Japanese men. Biochem Biophys Res Commun 1999;258:138–40.
22. Comings DE, Flanagan SD, Dietz G, Muhleman D, Knell E, Gysin R. The dopamine D2 receptor (DRD2) as a major gene in obesity and height. Biochem Med Metab Biol 1993;50:176–85.
23. Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID. Association of the TNF-alpha –308 G/A promoter polymorphism with insulin resistance in obesity. Obes Res 2002;10:401–7.
24. Chouchane L, Danguir J, Beji C, Bouassida K, Camoin L, Sfar H, et al. Genetic variation in the stress protein hsp70-2 gene is highly associated with obesity. Int J Obes Relat Metab Disord 2001;25:462–6.
25. Yanagisawa K, Hingstrup Larsen L, Andersen G, Drivsholm T, Cederberg A, Westergren R, et al. The FOXC2 –512C→T variant is associated with hypertriglyceridaemia and increased serum C-peptide in Danish Caucasian glucose-tolerant subjects. Diabetologia 2003;46:1576–80.
26. Brouillette C, Bosse Y, Perusse L, Gaudet D, Vohl MC. Effect of liver fatty acid binding protein (FABP) T94A missense mutation on plasma lipoprotein responsiveness to treatment with fenofibrate. J Hum Genet 2004;49:424–32.
27. Schalin-Jantti C, Valli-Jaakola K, Oksanen L, Martelin E, Laitinen K, Krusius T, et al. Melanocortin-3-receptor gene variants in morbid obesity. Int J Obes Relat Metab Disord 2003;27:70–4.
28. Acton S, Osgood D, Donoghue M, Corella D, Pocovi M, Cenarro A, et al. Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. Arterioscler Thromb Vasc Biol 1999;19:1734–43.
29. Ma X, Bacci S, Mlynarski W, Gottardo L, Soccio T, Menzaghi C, et al. A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. Hum Mol Genet 2004; 13:2197–205.
30. Elosua R, Demissie S, Cupples LA, Meigs JB, Wilson PW, Schaefer EJ, et al. Obesity modulates the association among APOE genotype, insulin, and glucose in men. Obes Res 2003;11:1502–8.
31. Carpenter KJ. A short history of nutritional science: part 3 (1912–1944). J Nutr 2003;133:3023–32.
32. World Health Organization: WHO Expert Committee on Diabetes Mellitus. Second Report. Geneva, WHO, 1980.
33. Coates PA, Ollerton RL, Luzio SD, Ismail I, Owens DR. A glimpse of the ''natural history'' of established type 2 (non-insulin dependent) diabetes mellitus from the spectrum of metabolic and hormonal responses to a mixed meal at the time of diagnosis. Diabetes Res Clin Pract 1994;26:177–87.
34. Igarashi M, Hirata A, Yamauchi T, Yamaguchi H, Tsuchiya H, Ohnuma H, et al. Clinical utility and approach to estimate postprandial hypertriglyeridcemia by a newly designed oral fat-loading test. Atheroscler Thromb 2003;10:314–20.
35. Marcoux C, Hopkins PN, Wang T, Leary ET, Nakajima K, Davignon J, et al. Remnant-like particle cholesterol and triglyceride levels of hypertriglyceridemic patients in the fed and fasted state J Lipid Res 2000;41:1428–36.
36. Harano Y, Miyawaki T, Nabiki J, Shibachi M, Adachi T, Ikeda M, et al. Development of cookie test for the simultaneous determination of glucose intolerance, hyper insulinemia, insulin resistance and postprandial dyslipidemia. Endocr J 2006;53:173–80.
37. Poitout V, Hagman D, Stein R, Artner I, Robertson RP, Harmon JS. Regulation of the insulin gene by glucose and fatty acids. J Nutr 2006;136:873–6.
38. Perez-Martinez P, Perez-Jimenez F, Bellido C, Ordovas JM, Moreno JA, Marin C, et al. A polymorphism exon 1 variant at the locus of the scavenger receptor class B type I (SCARB1) gene is associated with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet. J Clin Endocrinol Metab 2005;90:2297–300.

39. Olivieri O, Martinelli N, Sandri M, Bassi A, Guarini P, Trabetti E, et al. Apolipoprotein C-III, n-3 polyunsaturated fatty acids, and ''insulin-resistant'' T-455C APOC3 gene polymorphism in heart disease patients: example of gene-diet interaction. Clin Chem 2005;51:360–7.
40. Salas J, Jansen S, Lopez-Miranda J, Ordovas JM, Castro P, Marin C, et al. The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an oral-glucose-tolerance test after consumption of a diet rich in saturated fats Am J Clin Nutr 1998;68:396–401.
41. Wansink B, Westgren RE, Cheney MM. Hierarchy of nutritional knowledge that relates to the consumption of a functional food. Nutrition 2005;21:264–8.

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