Brief Genetics Report

The Common −866 G/A Polymorphism in the Promoter of Uncoupling Protein 2 Is Associated With Increased Carbohydrate and Decreased Lipid Oxidation in Juvenile Obesity

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Uncoupling protein (UCP) 2 is a member of the mitochondrial transporter superfamily that uncouples proton entry in the mitochondrial matrix from ATP synthesis. Although its physiological role remains to be established, UCP2 is considered a candidate gene for association with energy metabolism and obesity. A common promoter polymorphism, −866 G/A, has been associated with increased UCP2 gene expression and middle-aged adult obesity. In fact, our analysis of 296 juvenile obese and 568 nonobese control subjects revealed no difference in the prevalence of this polymorphism. Insulin and glucose response to oral glucose was comparable across the −866 genotypes. Metabolic studies in 147 of these juvenile obese subjects showed that homozygosity for the UCP2 promoter variant A was associated with important changes in energy metabolism compared with other genotypes, i.e., a 34% increase of carbohydrate oxidation (94 ± 10 vs. 70 ± 3 mg · min^{-1} · m^{-2}, $P = 0.004$) and a 23% decrease of lipid oxidation (26 ± 3 vs. 34 ± 1 mg · min^{-1} · m^{-2}, $P = 0.03$). Therefore, the juvenile obese subjects who are homozygous for the A variant have an increased ratio (3.6 ± 1.2) of calories derived from carbohydrates to those from lipids compared with G/A or G/G obese children (1.4 ± 0.2, $P = 0.003$), suggesting a role for UCP2 in the partitioning of metabolic fuels. *Diabetes* 53:235–239, 2004

The epidemic of childhood obesity that is currently taking over many parts of the world will contribute to a large increase of adult obesity and metabolic complications. It is thus important to study the early metabolic alterations characterizing juvenile obesity and the associated genetic factors. Because of its suspected function, the uncoupling protein (UCP) 2 is one of the many genes that might be associated with early obesity or with obesity effects on metabolic tissues. The human UCP2 gene encodes a mitochondrial transmembrane carrier protein that shows high amino acid similarities with the other two human UCPs, UCP1 and -3. UCP3 is predominantly expressed in skeletal muscle (1), but UCP2 appears to be widely expressed (2–4). UCP2 and UCP3 are located within 150 kb of each other on chromosome 11q13 (5,6). As a potential uncoupler of oxidative phosphorylation, UCP2 could be expected to play a role in energy balance and the regulation of body weight. However, it should be kept in mind that the physiological function of UCP2 is still a matter of debate and research (3). A number of genetic studies have investigated the relationship between the UCP2 locus and obesity propensity, with a specific focus on coding variants of this protein (7). Esterbauer et al. (8) recently reported that a functional G/A polymorphism in the promoter of UCP2 is associated with obesity in middle-aged European adults. The variant A allele of the −866 polymorphism was associated with enhanced transcription of the UCP2 gene and a decreased risk of obesity (8).

Since a large proportion of adult obesity starts during childhood (9,10), we examined whether the decreased prevalence of allele A seen in obese adults is already observable in juvenile obesity. The distribution of −866 G/G (0.385), G/A (0.490), and A/A (0.125) genotypes was in fact comparable in the 296 juvenile obese subjects (Table 1) and in the 568 never obese control subjects (not shown). The −866 polymorphism fulfilled Hardy-Weinberg expectations in both cohorts. Genotype prevalence was exactly similar to that found in the nonobese Caucasians by Esterbauer et al. (8). Age, sex, and BMI did not differ significantly between −866 genotypic groups of obese children (Table 1). These observations suggest that genomic variations nearby the UCP2 gene do not play a major role in the predisposition to juvenile obesity, in contrast with middle-aged obese adults, who show an increased proportion of G/G genotypes (8). Our interpretation is that juvenile forms of obesity studied here may not share all of their genetic factors with the late adulthood forms studied by Esterbauer et al. (8) ("early-onset obesity" versus "late-onset obesity"). According to this view, the −866 G allele would predispose an increased proportion of obese juveniles to maintain their obesity status during adulthood.

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OGTT, oral glucose tolerance test; UCP, uncoupling protein.

TABLE 1
Distribution of UCP2 genotypes and main characteristics in the whole cohort of 296 obese children

UCP2 genotype	−866 G/G	−866 G/A	−866 A/A
n	114	145	37
Prevalence	0.385	0.490	0.125
Male/female	51/63	54/91	19/18
Age (years)	12.1 ± 0.3	11.7 ± 0.2	11.8 ± 0.5
BMI (kg/m^2)	29.6 ± 0.5	29.6 ± 0.4	29.3 ± 0.8

Data are means ± SE.

UCP2 is a negative regulator of insulin secretion (11) and could thus be a link between obesity and β-cell dysfunction in obesity-induced type 2 diabetes (11). Alterations in fatty acid metabolism are likely involved in the mechanisms of this β-cell dysfunction (11). Because of the clinical (12,13) and experimental (14) association of β-cell UCP2 expression with insulin secretion (15), we searched for effects of −866 G/A polymorphism on the insulin response to glucose by performing oral glucose tolerance tests (OGTTs) in 129 obese children. We found that fasting glucose, fasting insulin, and insulin and glucose areas did not differ between the −866 genotypic groups (Fig. 1 and Table 2). These findings support the theory that glucose-

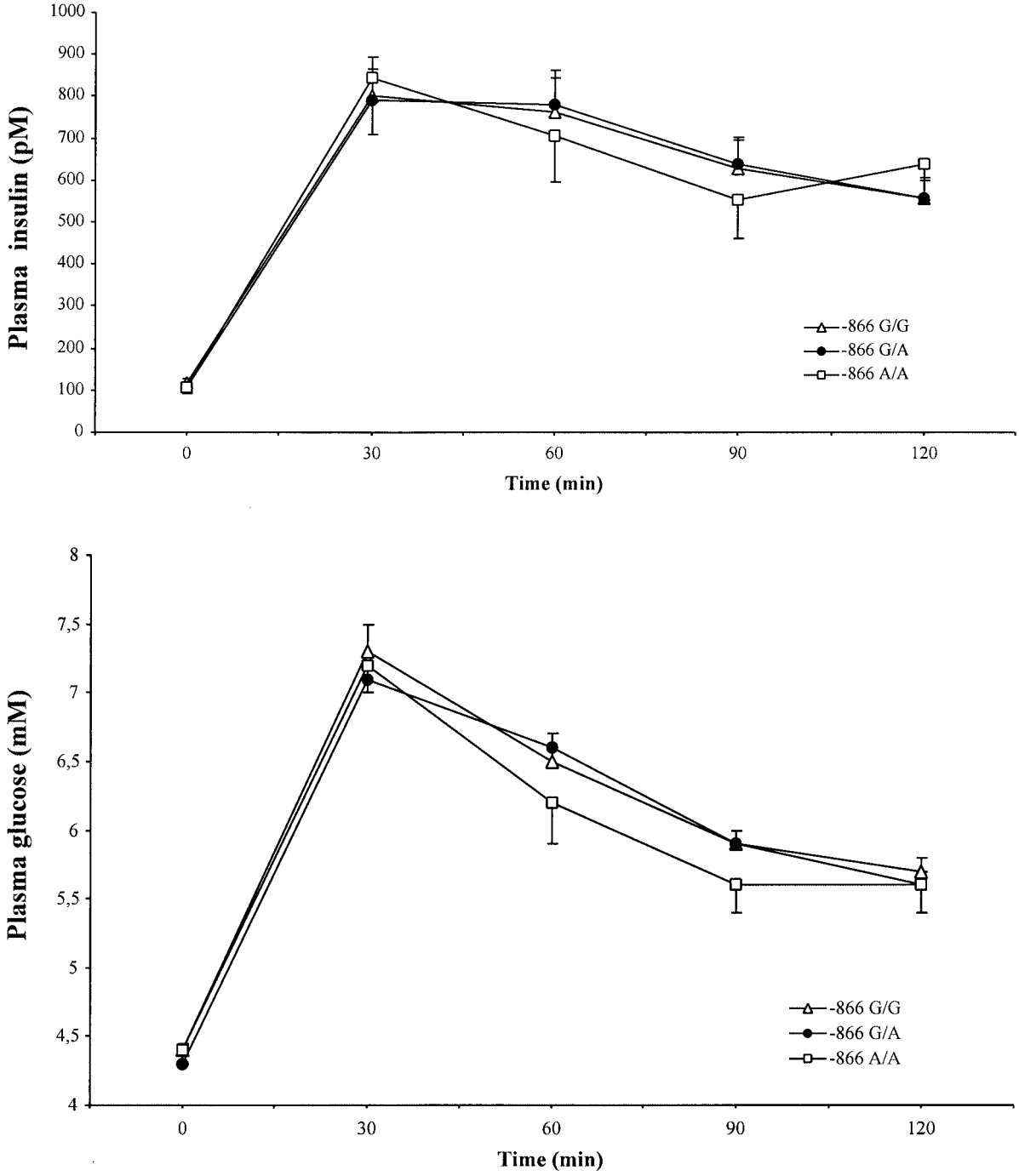

FIG. 1. Plasma insulin and glucose during OGTT in the 129 obese children according to their −866 UCP2 genotype.

TABLE 2
Glucose and insulin parameters during OGTT in a subgroup of obese children; a group of age-matched nonobese children is presented for comparison

UCP2 genotype	Obese children			Lean children
	GG	GA	AA	
n	46	68	15	38
Male/female	22/24	28/40	6/9	18/30
Age (years)	12.1 ± 0.4	11.7 ± 0.3	11.6 ± 0.9	11.1 ± 0.6
BMI (kg/m^2)	30.0 ± 0.8	30.1 ± 0.6	30.2 ± 1.6	16.6 ± 0.2*
Fasting insulin (pmol/l)	117 ± 9	106 ± 7	107 ± 15	57 ± 2*
Fasting glucose (mmol/l)	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
Insulin area ($\times 10^3$ pmol/l)	76 ± 7	76 ± 7	74 ± 10	28 ± 2*
Glucose area (mmol/l)	744 ± 13	737 ± 12	724 ± 17	700 ± 14

Data are means ± SE. *$P < 0.001$ vs. the whole cohort of 129 obese children.

stimulated insulin secretion is independent of the −866 UCP2 genotype in young obese patients (16,17). In addition, the close similarity of insulin and glucose curves across genotypes during OGTT (Fig. 1) indicates that the −866 variants of the UCP2 promoter are not associated with variations in insulin sensitivity at the whole-body level. In middle-aged adults, the −866 A/A genotype was found to be associated with slightly higher fasting insulin levels (8) but with a decreased acute insulin response to intravenous glucose (18). None of these genotypic differences, however, were of sufficient magnitude to reach significance (8,18). It is possible that, during evolution from adolescence to late adulthood, the −866 A allele of the UCP2 promoter is associated with a diminution of insulin secretion, which could have two effects. One would be to reduce the insulin-dependent deposition of fat and thus to account for the decreased prevalence of middle-age obesity observed in A/A homozygous subjects (18). The other would be to increase the risk of type 2 diabetes in these individuals (18).

We previously reported that postabsorptive energy metabolism in juvenile obese subjects is characterized by an early increase in lipid oxidation followed by a progressive decrease in glucose oxidation (19). Obese children derive ~61% of their energy production from lipids compared with 34% in lean children. The present results show that the −866 G/A polymorphism is associated with changes in the rates of oxidation of carbohydrate and fatty acid fuels in the current cohort of young obese subjects. Obese children with −866 A/A genotypes have a modest but significant increase of resting energy expenditure, possibly consistent with the decreased risk of middle-age obesity observed in these subjects by Esterbauer et al. (8). Astrup et al. (20) and Ukkola et al. (21) both reported that variations in lipid fuel oxidation, reflected by respiratory quotient measurement, is associated with the A/V polymorphism of UCP2 protein in healthy adults. We also observed that despite comparable glucose, free fatty acid, and insulin levels, the obese children with −866 A/A genotypes oxidize more glucose and less lipids than the −866 G/G or G/A groups (Table 3). We have no direct explanation for this phenomenon. According to a recent hypothesis (22), UCP2 and -3 may play a role in the export of fatty acids from mitochondria (23) when fatty acid oxidation predominates. An increase of UCP2 expression would thus be associated with an increase of fatty acid oxidation. However, if UCP2 is truly an uncoupling molecule, it should instead favor an increase of intracellular ATP, an increase of glucose oxidation, and a decrease of lipid oxidation ("Pasteur" effect). In obese subjects, most of glucose and lipid oxidation takes place in the skeletal muscle, a tissue in which ethics do not allow us to measure

TABLE 3
Main clinical and metabolic characteristics in the UCP2 genotypic subgroups of 147 obese children and in 17 age-matched nonobese children.

UCP2 genotype	Obese children			Lean children
	−866 G/G	−866 G/A	−866 A/A	
n	55	75	17	14
Male/female	21/34	30/45	12/5	7/7
Age (years)	11.7 ± 0.3	11.5 ± 0.3	10.9 ± 0.6	11.9 ± 0.7
BMI (kg/m^2)	28.8 ± 0.7	29.6 ± 0.6	27.6 ± 0.7	18.1 ± 0.8*
Fasting glucose (mmol/l)	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.1	4.3 ± 0.1
Fasting insulin (pmol/l)	88 ± 6	98 ± 7	79 ± 6	54 ± 4*
Free fatty acid (mmol/l)	0.61 ± 0.04	0.58 ± 0.03	0.53 ± 0.05	0.51 ± 0.10
Non protein respiratory quotient	0.844 ± 0.010	0.838 ± 0.070	0.865 ± 0.016	0.900 ± 0.020
Resting energy expenditure/lean body mass	43 ± 1†	49 ± 2	55 ± 8	46 ± 4*
Glucose oxidation rate ($mg \cdot m^{-2} \cdot min^{-1}$)	71 ± 4	69 ± 3	94 ± 10‡	136 ± 6*
Lipid oxidation rate ($mg \cdot m^{-2} \cdot min^{-1}$)	33 ± 2	34 ± 1	26 ± 3‡	25 ± 5*
Calories from glucose/calories from lipids	1.75 ± 0.41	1.23 ± 0.18	3.59 ± 1.23§	5.45 ± 0.55*

Data are means ± SE. *$P < 0.01$ vs. the whole cohort of 147 obese children; †$P < 0.05$ vs. G/A, $P < 0.03$ vs. A/A; ‡$P < 0.005$ vs. G/A, $P < 0.02$ vs. G/G; §$P < 0.001$ vs. G/A, $P = 0.07$ vs. G/G.

UCP2 gene expression. It is possible that −866 G/A genotypes are associated with changes in UCP2 expression in muscle comparable with those observed in the adipose tissue of obese adults with this genotype (8). In obese adults, however, the expression of the UCP2 gene shows discordant changes in muscle and adipose tissue (24–28). It is also notable that UCP2 expression seems to be much less widespread as a protein than as mRNA species (3,4) and that most experimental and clinical reports on UCP2 expression levels are based only on mRNA measurements.

In summary, the −866 G/A polymorphism in the UCP2 promoter seems to underlie modifications in the fluxes of substrates that are oxidized in the mitochondria of young obese patients. Randle et al. (29) proposed the possibility that the impairment in glucose metabolism that characterizes obese subjects is caused by an excessive utilization of fatty substrates. The conditions on which Randle et al.'s hypothesis can hold in clinical situations are *1*) lipid oxidation is increased, *2*) glucose oxidation is decreased, and *3*) the two changes are commensurate to one another. Our observations with UCP2 genotypes suggest that UCP2 could play a significant role in the regulation of the phenomenon of Randle et al. in young obese subjects. Commonly, genetic association studies try to interpret positive results in the light of the physiology of the considered molecule. In the case of UCP2, such an attempt should remain very cautious since the precise role of this carrier protein in mitochondrial lipid metabolism remains to be defined. On the other side, further epidemiogenetic studies will be necessary to determine whether the −866 UCP2 promoter polymorphism influences insulin secretion and substrate oxidation during evolution from juvenile to late-adulthood obesity and if it is truly implicated in the natural history of obesity-dependent diabetes.

RESEARCH DESIGN AND METHODS

The 296 obese patients came from a previously described cohort (30) originating from Mediterranean and Central European countries. The geographic origin of the patients was assessed through family history, analysis of patronymic names, and grandparents' birthplace. Inclusion criteria were a BMI exceeding the 85th centile before the age of 6 years, a monotonic weight curve since birth, and the lack of any weight reduction during obesity course. There were 147 obese children from this cohort who underwent indirect calorimetry, and another group of 129 obese patients had OGTTs.

A total of 568 young adults aged 22–40 years, of comparable Caucasian origin (30), and who had never been obese, were studied as control subjects.

The obese and lean children were genotyped at position −866 of the UCP2 locus as reported (31). Single nucleotide polymorphism genotypes were determined by the analysis of PCR products.

To minimize experimental variability in measuring insulin levels, we used codified conditions. After 12 h of overnight fasting following 3 days of standardized diet (caloric and carbohydrate content) in hospital, plasma insulin was measured in unstressed conditions (an intravenous microcatheter was placed in a peripheral vein 24 h before sampling) (30). The OGTT consisted of the ingestion of 1.75 g/kg glucose (75 g maximum) in 200 ml lemon-flavored water at 10°C and venous blood sampling at 0, 30, 60, 90, and 120 min after the end of the ingestion. Plasma insulin was measured in each sample in duplicate with standard radioassay (30). As a reproductibility test, duplicate OGTT measurements were performed on following days in 20 children. The intraindividual coefficient of variation of insulin values at the various time points was 13–18%.

We used insulin (μU/ml) and glucose (mg/dl) values during the OGTT to calculate the composite whole-body index insulin sensitivity index (ISI_{comp}) described by Matsuda and DeFronzo (32), according to the formula ISI_{comp} = 10,000/square root of (Ins 0 × Glu 0 × mean OGTT Ins × mean OGTT Glu).

Respiratory calorimetry, energy expenditure, resting metabolic rate, percent body fat, glucose and fat oxidation, and lean body mass were measured as previously described (33).

All data are presented as means ± SE. The statistical comparisons used ANOVA among the various subgroups.

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