

Fig. 1. IEF and CE profiles of serum Tf extracted from samples spotted on Guthrie cards.

(A), spotting of whole blood may identify CDG patients (lane 2), may give doubtful results when the protein concentration is low (lane 3), or may give background disturbance when samples are concentrated (lane 4). Lane 1, control. (B), spotting of serum permits identification of control (lane 1), CDG-I (lane 2), and CDG-II (lane 3) sera by IEF. Guthrie cards are better stored at -20°C (lanes F) than at room temperature (lanes R). (A and B), numbers on the left indicate sialo-Tf fractions. (C), CE permits identification of control (trace 1), CDG-I (trace 2), and CDG-II (trace 3) samples extracted from serum-spotted Guthrie cards.

cal differences, in both IEF and CE, between serum and serum spotted on Guthrie cards nor differences between the Tf profiles on CE. We therefore expect that the rate of positive outcomes suggesting CDG will be similar.

In conclusion, analysis of a sufficient amount of serum spotted on a Guthrie card, stored at -20°C and transported by air, gives information about the Tf profile similar to that obtained by analysis of serum. Although serum samples are preferable, we recommend the present procedure if Guthrie cards are used.

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References

1. Jaeken J, van Eijk HG, van der Heul C, Corbeel L, Eeckels R, Eggemont E. Sialic acid-deficient serum and cerebrospinal fluid transferrin in a

newly recognized genetic syndrome. *Clin Chim Acta* 1984;144:245–7.

2. Helander A, Bergström J, Freeze HH. Testing for congenital disorders of glycosylation by HPLC measurement of serum transferrin glycoforms. *Clin Chem* 2004;50:954–8.
3. Carchon HA, Chevigné R, Falmagne JB, Jaeken J. Diagnosis of congenital disorders of glycosylation by capillary zone electrophoresis of serum transferrin. *Clin Chem* 2004;50:101–11.
4. Stibler H, Cederberg B. Diagnosis of the carbohydrate-deficient glycoprotein syndrome by analysis of transferrin in filter paper blood spots. *Acta Paediatr* 1993;82:55–9.
5. Carchon H, Serrus M, Eggemont E. Digestion of gliadin peptides by intestinal mucosa from control or coeliac children. *Digestion* 1979;19:1–5.

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Prenatal Diagnosis of Guanidinoacetate Methyltransferase Deficiency: Increased Guanidinoacetate Concentrations in Amniotic Fluid

To the Editor:

Guanidinoacetate methyltransferase (GAMT; EC 2.1.1.2) deficiency (OMIM 601240) is an autosomal recessive disorder of creatine biosynthesis, characterized clinically by mental retardation, language delay, extrapyramidal movements, epilepsy, and autistic behavior (1). Biochemically, GAMT deficiency is characterized by depletion of creatine and accumulation of guanidinoacetate (GAA) in the brain and body fluids (2). Treatment by creatine supplementation (combined with arginine restriction and ornithine supplementation) partially restores (~70%) cerebral creatine, reduces seizures, and improves behavior, but it does not reverse the mental retardation (3). We have described a method to measure GAA and creatine in plasma and urine by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (4). In the present study, we validated this method for measurement of GAA and creatine in amniotic fluid, and we report the first GAMT prenatal diagnosis based on a combination of molecular and biochemical investigations.

We adapted a previously reported method to measure GAA and creatine in plasma (4) for amniotic fluid. Briefly, 50 μL of supernatant of amniotic fluid or aqueous calibrators were mixed with internal standards { d_3 -creatine (CDN isotopes) and [$^{13}\text{C}_2$]GAA (Dr. Ten Brink, VU University Medical Center, Amsterdam, The Netherlands)}. Samples were ex-

Table 1. Reference intervals for GAA and creatine in amniotic fluid and results obtained for affected pregnancy.

Gestational age ^a	GAA, $\mu\text{mol/L}$		Creatine, $\mu\text{mol/L}$	
	Mean (SD)	Range	Mean (SD)	Range
All ages (n = 82)	2.62 (0.50)	1.55–4.43	54.7 (11.6)	28.0–88.3
15 WA (n = 10)	2.96 (0.70)	2.14–4.43	59.7 (9.2)	49.8–79.6
16 WA (n = 21)	2.67 (0.46)	2.04–4.06	56.9 (9.6)	43.1–76.7
17 WA (n = 21)	2.56 (0.41)	1.66–3.48	54.0 (12.9)	28.0–88.3
18 WA (n = 15)	2.63 (0.49)	1.93–3.53	52.6 (12.7)	29.5–69.4
19 WA (n = 10)	2.40 (0.52)	1.55–3.62	48.1 (10.8)	30.5–65
20 WA (n = 5)	2.46 (0.25)	2.27–2.87	57.2 (14.5)	41.9–75.6
Affected fetus (15 WA)		11.43		48.4

^a Gestational ages are given in weeks of amenorrhea (WA).

tracted and derivatized as butyl esters. LC-MS/MS analysis was performed in the multiple-reaction monitoring mode (API 2000; Sciex Applied Biosystems). Calibration curves were constructed by linear regression analysis of the ratios of the GAA and creatine calibrators to their respective internal standards.

The intraday imprecision (CV) of the global procedure (extraction and quantification) was estimated on a pool of amniotic fluids extracted 10 times and injected; the CVs were 9% for GAA and 5% for creatine. Interday imprecision was assessed in the same pool, extracted and injected on 5 different days; the CVs were 7% for GAA and 5% for creatine. For recovery, we added 2 concentrations of GAA and creatine (n = 5 for each concentration) to pooled amniotic fluid. The recoveries of 10 and 20 $\mu\text{mol/L}$ GAA were 94% (CV = 4%) and 106% (CV = 3%), respectively, and the recoveries of 100 and 200 $\mu\text{mol/L}$ creatine were 88% (CV = 3%) and 98% (CV = 2%), respectively.

We established control values by measuring GAA and creatine in 82 amniotic fluid samples from women at risk for carrying a fetus with trisomy 21; samples were obtained by amniocentesis between 15 and 20 weeks of amenorrhea. In all cases, the karyotype was normal. The results are given in Table 1.

We used this method to determine a prenatal diagnosis in a family with a 10-year-old child affected with GAMT deficiency. The diagnosis of GAMT deficiency was based on in-

creased urinary concentrations of GAA and was confirmed by mutational analysis of the *GAMT* gene. A novel homozygous missense mutation was detected in exon 1 (c.148A>C; p.Met50Leu), which affected a highly conserved amino acid. The parents were heterozygous for this mutation. At the time of diagnosis of the index patient, the mother was pregnant, and amniocentesis was performed at 15 weeks of amenorrhea for prenatal diagnosis of GAMT deficiency by screening for the familial mutation in amniocytes and GAA measurement in amniotic fluid.

Assay results (Table 1) indicated increased GAA (11.4 $\mu\text{mol/L}$) and normal creatine (48 $\mu\text{mol/L}$). The fetus was homozygous for the familial mutation, confirming the biochemical diagnosis of GAMT deficiency. The parents decided—partly because of the lack of experience with treatment at early age—to terminate the pregnancy.

We have validated a rapid method to measure GAA and creatine in amniotic fluid and have determined expected values at 15–20 weeks of amenorrhea. The feasibility of GAA measurement in amniotic fluid by gas chromatography–mass spectrometry has been reported (5). Here we describe the first prenatal diagnosis confirming that increased GAA in amniotic fluid appears to be pathognomonic for GAMT deficiency. Measurement of GAA in amniotic fluid may therefore be useful in the prenatal diagnosis of GAMT deficiency.

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References

1. Stromberger C, Bodamer OA, Stockler-Ipsiroglu S. Clinical characteristics and diagnostic clues in inborn errors of creatine metabolism. *J Inher Metab Dis* 2003;26:299–308.
2. Carducci C, Birarelli M, Leuzzi V, Battini R, Cioni G, Antonozzi I. Guanidinoacetate and creatine plus creatinine assessment in physiologic fluids: an effective diagnostic tool for the biochemical diagnosis of arginine:glycine amidinotransferase and guanidinoacetate methyltransferase deficiencies. *Clin Chem* 2002;48:1772–8.
3. Schulze A, Ebinger F, Rating D, Mayatepek E. Improving treatment of guanidinoacetate methyltransferase deficiency: reduction of guanidinoacetic acid in body fluids by arginine restriction and ornithine supplementation. *Mol Genet Metab* 2001;74:413–9.
4. Cognat S, Cheillan D, Piraud M, Roos B, Jakobs C, Vianey-Saban C. Determination of guanidinoacetate and creatine in urine and plasma by liquid chromatography–tandem mass spectrometry. *Clin Chem* 2004;50:1459–61.
5. Struys EA, Jansen EE, ten Brink HJ, Verhoeven NM, van der Knaap MS, Jakobs C. An accurate stable isotope dilution gas chromatographic–mass spectrometric approach to the diagnosis of guanidinoacetate methyltransferase deficiency. *J Pharm Biomed Anal* 1998;18:659–65.

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Smoking Acutely Increases Plasma Ghrelin Concentrations

To the Editor:

Smoking and smoking cessation are associated with weight changes (1), and many smokers give weight gain as a primary reason for not trying to quit smoking (2). A relationship has been reported between smoking and plasma concentrations of ghrelin, a 28-amino acid growth hormone-releasing peptide secreted mainly by the stomach (3–5). Our primary aim was to determine whether smoking has an acute effect on plasma ghrelin concentrations.

We enrolled 36 healthy volunteers, 28 men [mean (SD) age, 28.4 (7.5) years] and 8 women [30.4 (9.8) years]; 26 volunteers were smokers and 10 were nonsmokers. The study was approved by the Ethics Committee of our institution, and informed, written consent was obtained from each participant. None of the individuals had a history of gastric surgery or any serious health problems.

Smoking history and body mass index (BMI) were determined for all participants. Blood samples were collected after overnight fasting, and plasma was frozen in aliquots at -80°C immediately after centrifugation (1600g for 15 min at 4°C).

We measured ghrelin with an enzyme immunoassay (Phoenix Pharmaceuticals). The intraassay CV was $<5\%$, the interassay CV was $<14\%$, and the lower detection limit was $0.1\ \mu\text{g/L}$. The participants smoked 1 filtered cigarette containing 0.8 mg of nicotine under highly regulated conditions. Every 15 s, a puff lasting 5 s was taken, and the whole cigarette had to be smoked within 5 min. Plasma ghrelin concentrations were determined at 0, 2, 5, 15, and 60 min after the initiation of smoking.

We used the Student *t*-test to compare the mean values for the studied groups. Significance between groups was set at $P = 0.05$. The mean (SD) plasma concentrations for baseline and at 2, 5, 15, and 60 min are shown in Fig. 1. We observed significant increases ($P < 0.001$) from baseline at 2, 5, and 15 min after smoking. A separate analysis revealed that increases occurred in both smokers and nonsmokers. In general, peak values were observed at 2 min (Fig. 1). Baseline mean values for smokers did not differ significantly from those for nonsmokers. We observed no significant difference in mean (SD) BMI between men [$25.8 (2.5)\ \text{kg/m}^2$] and women [$23.4 (5.1)\ \text{kg/m}^2$; $P = 0.73$] or between smokers [$25.8 (3.2)\ \text{kg/m}^2$] and nonsmokers [$23.7 (3.3)\ \text{kg/m}^2$; $P = 0.1$].

This study demonstrates the acute

effect of cigarette smoking on plasma concentrations of the novel orexigenic hormone ghrelin. The most interesting finding of this study was the unexpected increase in ghrelin concentrations shortly after smoking. The effect of smoking 1 cigarette continued for at least 15 min and was independent of the BMI of each individual. The most intense increase was noted 2 min after smoking initiation. Given the known anorexic action of smoking (6), a decrease in ghrelin concentrations might be expected as an acute effect of smoking. Alcohol has also exhibited an unexpected acute inhibitory effect on ghrelin secretion in healthy individuals, despite its strong orexigenic influence in humans (7).

We speculate that this acute increase of ghrelin during smoking is related to adverse effects on gastric mucosa, gastric motility, mucosal blood flow, and concentrations of free radicals (8). Enhancement of ghrelin secretion could also be an indirect influence mediated by other factors, including growth hormone (nicotine increases the growth hormone concentration) (9), leptin (inverse correlation with ghrelin concentrations) (10), and vagal nerve stimulation (smoking affects vagal nerve activity) (11). Our finding that smoking is more likely to produce an acute release of intracellular ghrelin into the circulation rather than a time- or dose-dependent stomach secretion is partially in agreement with the hypothesis of investigators (4, 5) that circulating ghrelin concentrations are related to the time elapsed after smoking. Rather than being de-

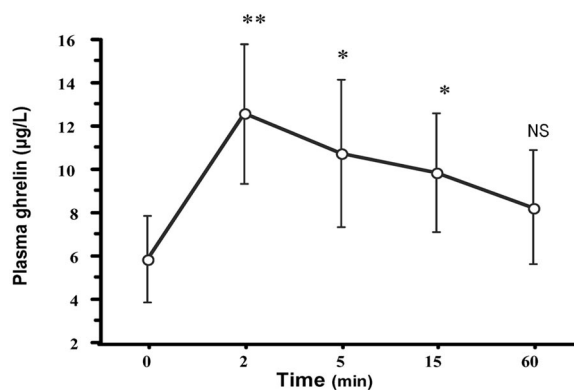


Fig. 1. Mean (SD) plasma ghrelin concentrations ($\mu\text{g/L}$) after cigarette smoking for all study participants.

** $P = 0.0005$; * $P < 0.001$; NS, not significant.