

hypothalamopituitary unit. Ho suggests that this test can safely establish GH deficiency only in patients with organic pituitary disease. Popovic and colleagues should, therefore, reanalyse their data after dividing their patients into those with known hypothalamic disease and those with organic disease.

\*D Devendra, R Williams, T J Wilkin

\*University Medicine and Derriford Combined Laboratory, Derriford Hospital, Plymouth, PL6 8DH, UK; and Peninsula Medical School, Plymouth (e-mail: senan.devendra@phnt.swest.nhs.uk)

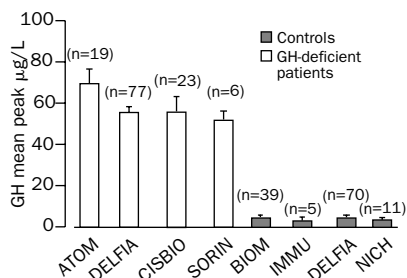
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#### Authors' reply

Sir—We agree with D Devendra and colleagues that the diagnosis of GH deficiency in adults is complex, since several variables affect the outcome of GH-provocative tests.

The advantage of the combined administration of GHRH-GHRP-6, which we propose as the new gold standard test for GH reserve in adults, is that in addition to its being devoid of side-effects, it is not affected by age, sex, adiposity, thyroid status, glucocorticoid status, diabetes mellitus, time of day, previous food intake, pre-treatment with GH, or the previous GH basal concentrations.<sup>1</sup> Moreover, to have a test not affected by the assay used would be a bonus, since the assay is a powerful factor that confuses the diagnosis of GH deficiency.

As proof of absence of interaction, the ITT cut-off of 3 ng/mL is valid



#### GH mean peak in controls and GH-deficient patients with different assays

BIOM=BioMérieux, IMMU=Immunitel, NICH=Nichols.

only for polyclonal antibody-based assays,<sup>1</sup> which have almost disappeared from current laboratory use. For the most commonly used test, therefore, there is no widely accepted valid cut-off value.<sup>2</sup> We are aware of the systematic bias introduced in the GH determination by the assay used, and we agree that we have not done a rigorous analysis of how the GH peaks produced by the combined test are altered by different assays, since this analysis was out of the scope of the study. However, in our preliminary data, not included in the report because of space constraints, we found that mean GH peaks in the different assays were similar (figure), although the number of patients in each group was too different to be sure. Second, when we took into account only the values provided by Delfia (time-resolved monoclonal-antibody-based fluoroimmunoassay), the result was identical. These data suggest that the noise introduced by use of seven different assays in the same study was not intense enough to alter the identification of healthy patients and those with GH deficiency.

GHRH-GHRP-6 test does not act at the pituitary level. Although GH secretagogues were developed by their in-vitro GH-releasing capabilities, overwhelming evidence shows that compounds such as GHRP-6 and the combined GHRH-GHRP-6 act mainly at the hypothalamus.<sup>1</sup> We, along with other groups, have reported that any organic alteration in the hypothalamus, the pituitary stalk, or the pituitary, blunts GHRH-GHRP-6-mediated GH release.<sup>3</sup> Therefore, the test explores the whole hypothalamopituitary unit, as does the ITT, identifying any organic or structural alteration. The efficacy of the test in the so-called functional, non-organic or idiopathic GH deficiencies has not yet been assessed. However, several groups agree that this syndrome, which is fairly rare in adults, is debatable or at least poorly defined.

The GHRH-GHRP-6 test is scarcely affected by the GH assay used and detects organic lesions located at any level of the hypothalamopituitary somatotrope axis. We eagerly await the experience of other groups with this test in the clinical setting.

V Popovic, C Dieguez, \*F F Casanueva, on behalf of all authors

Institute of Endocrinology, Belgrade Yugoslavia; Department of Endocrinology, Academisch Ziekenhuis, Utrecht, Netherlands; Vigo, Sevilla and Granada, Spain; and \*Department of Physiology and Medicine, PO Box 563, Santiago de Compostela E-15780, Spain

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#### Rats and risk

Sir—Research has shown that the so-called natural pesticide, rotenone, might be associated with Parkinson's disease.<sup>1,2</sup> As the news began to slowly circulate, the saying by Victor Cohn (a once senior columnist with the Washington Post) that "Scientists are to journalists what rats are to scientists" came to mind. The research in question showed that rotenone can produce Parkinson's disease in rats when it is administered via injection in low doses. Most rats, and human beings, however, do not willingly undertake direct injections of any sort of pesticide, natural or not. So the results and their applicability to human health remain controversial. But, rates are one of the, albeit blurry, windows on long-term human health effects. So the risk question that arises is, are natural pesticides potentially dangerous?

In the autumn of 1998, Arpad Pusztai from the UK told television interviewers that a handful of rats fed genetically engineered compared with those fed conventional potatoes displayed some differences—differences that soon became a mantra for many around the globe, including journalists, as evidence of hypothetical danger associated with genetically engineered crops.

*The Lancet* also published Putztai's experiments<sup>3</sup> with the aim of "making constructive progress in the debate between scientists, the media, and the general public about the safety of GM foods".<sup>4</sup> On the other hand, the experiments flagging the possible dangers of rotenone, which has been marketed and used in the public domain for many decades as a so-called natural pesticide (sometimes used and marketed by the organic movement) and in various commercial garden care and animal-care products, barely stirred the interest of journalists.

Why was it that one story received so much more attention than the other? Was it that opponents of so-called genetically modified food (of who the loudest are frequently connected to the organic food movement) pushed and promoted the story for their own cause? After all, if conventional foods are deemed safe for people and the environment, then in the absence of a media flurry, why would consumers pay more for hypothetical benefits?

The same media forces that propelled Pusztai's rats to mainstream conversation have been largely silent when it comes to the rotenone rats. Since the organic movement uses rotenone itself, maybe they are choosing to remain quiet on this issue. Surely this action (or lack thereof) brings to light a severe case of double standards. For example, we have yet to see the Greenpeace press release condemning organic farmers for using rotenone and demanding the immediate removal of the roughly 680 rotenone-containing products from the supermarket shelves.

The latest findings about rotenone, which like Pusztai's results draw attention to the need for "further scientific attention", underscores a fundamental approach that North American regulators have taken to various products, including genetically-engineered foods: that is, that nature is not benign, and irrespective of the process used to create new foods—be it genetic engineering, conventional breeding, and a whole host of powerful techniques in between, the end product needs to undergo scientifically valid safety assessments.

The natural does not automatically mean safe. This premise proves the point made by Richard Horton that "What matters is what people believe about (these) risks and why the hold those beliefs".<sup>5</sup>

\*Shane Morris, Doug Powell

Department of Plant Agriculture, Crop Science Building, University of Guelph, Guelph, Ontario N1G 2W1, Canada

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## Prenatal identification of fetal genetic traits

Sir—Hiroshi Saito and colleagues (Sept 30, p 1170)<sup>1</sup> describe the prenatal diagnosis of a single-gene disorder without clinical consequences, in late pregnancy (fetal achondroplasia) by measurement of extracellular fetal DNA in maternal plasma. This report has once again raised hopes that reliable non-invasive prenatal investigation for fetal genetic loci has made the transition from the laboratory to the clinical arena. We caution, however, the premature introduction of this method into practice.

The basis for the approach originates from the observation made by Lo and colleagues<sup>2</sup> that free fetal DNA can be detected by PCR in maternal plasma or serum samples. Since fetal and maternal free DNA are present in the maternal circulation, previous studies have focused on the detection of fetal loci not present in the maternal genome, such as the Y chromosome or the rhesus D gene in rhesus d pregnant women.<sup>3,4</sup> Fetal DNA sequences are more readily detected in second and third trimester maternal blood samples than in those obtained early in pregnancy.<sup>3</sup> Diagnosis should, however, be confirmed by an independent test, such as ultrasonography, as Saito and colleagues used, because serious consequences, including termination, might depend on the in-utero findings.

To test the diagnostic accuracy and feasibility of Saito and colleagues' approach, we did a large-scale study of more than 200 samples. We used a highly sensitive real-time PCR technique, which proved suitable for the detection of free fetal loci.<sup>3</sup> Furthermore, since this technology is more amenable to automation and not as prone to contamination as is conventional PCR, it is therefore better suited for routine applications.

We have chosen to focus on the fetal rhesus D gene, which would be useful for diagnosis in pregnancies with a rhesus constellation, and on fetal sex, which is important to know in pregnancies at risk for X-linked disorders. In our experimental validation, done on plasma samples obtained from 22 normal healthy men and 48 non-pregnant women, no false results were recorded. The PCR assay for the rhesus D gene detected no anomalous results on plasma samples obtained from 24 rhesus d or 27 rhesus D individuals.

In 11 (6%) of 185 instances, however, in which the fetus was male,

no male free fetal DNA was detectable by the real-time PCR assay specific for the Y chromosome. All 52 samples obtained from pregnancies with female fetuses were identified correctly. The assay for the rhesus D gene could detect fetal rhesus D genotype correctly in 24 (96%) of 25 instances. In two (22%) of the nine pregnancies with a rhesus d fetus the fetus was incorrectly genotyped as being rhesus D.

The frequency of false-negative results for the two assays was close to 5%. Although free fetal DNA is less prevalent early in pregnancy than in the late second or third trimesters, our result cannot be attributed to this factor, since we took samples at 11.5–34.6 weeks' gestation (median of 16.5). In addition, some samples with no detectable concentrations of free fetal DNA had abundant quantities of free maternal DNA on PCR for the ubiquitous glyceraldehyde-3-phospho-dehydrogenase (GAPDH) gene. Therefore, false-negative results probably arose because of a technical deficit such as the inability to extract free DNA from the sample in question.

The two false-positive results for the rhesus D gene are probably attributable to contamination of the initial PCR template material, despite rigorous precautions.<sup>5</sup> Appropriate strategies are needed to ensure that no false-negative results arise in this way.

Our data suggest that, even though the assay is specific, because of the poor sensitivity (95%), the use of free fetal DNA from maternal plasma is currently not suitable for routine prenatal diagnosis of fetal genetic traits in a clinical setting, even with use of the most advanced methods currently available.

Xiao Yan Zhong, Sinuhe Hahn,  
\*Wolfgang Holzgreve

Department of Obstetrics and Gynaecology, University of Basel, CH-4031 Basel, Switzerland (e-mail: wholzgreve@uhbs.ch)

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