statistically significant effects (10 nmol/l) ($p < 0.05$) was considered to be low. It is well-known that hepatocytes degrade insulin [1, 2]. As far as we know, no data are available on the half-life of insulin in primary cultures of monkey hepatocytes. If we assume, however, that the insulin half-life in the latter hepatocyte cultures is similar to that in rat hepatocytes [3] and HepG2 cells [4], which is between 4 and 5 h in both cell models, 10 nmol/l is a relevant concentration. After 24 h of culture (the length of our incubation periods) about 2 % of the initial concentrations are left, being approximately 0.2 nmol/l. To be able to measure sufficient amounts of apolipoprotein(a) accumulating in the medium, we need to do overnight incubations. We choose incubation periods of 24 h, explaining the necessity for high initial concentrations compared with insulin concentrations in humans. We have reported previously that extinction of initially activated gene expression may occur during 24-h incubation periods without intermediate renewing of biologically active compounds which are heavily metabolised by hepatocytes [5]. As we have shown the suppressing effect of insulin (10 nmol/l) on apolipoprotein(a) expression was statistically significant ($p < 0.05$) after two 24-h periods, indicating that regulation is relatively slow. If on the other hand, metabolic effects of insulin proceed rapidly, i.e. within a few hours, lower initial insulin concentrations can be applied. This is the case in a report that the effect of insulin (0.6 nmol/l) on cholesterol removal from macrophages reaches a maximum after 4 to 6 h [6]. Similarly, e.g. mRNA expression of microsomal triglyceride transfer protein in HepG2 cells decreased within 4 to 6 h at 1 nmol/l insulin [7] and we found that 1.4 nmol/l insulin increased plasminogen activator inhibitor-1 activity and mRNA expression after 4 h in HepG2 cells and human hepatocytes [8]. In conclusion, results at high initial insulin concentrations are perhaps more physiologically relevant than thought and may reflect insulin effects as occur during peak portal concentrations (approximately 0.5 nmol/l).

The enzyme-linked immunoassay in our experiments (Tint-Elize, Biopool AB) uses polyclonal antibodies to human apolipoprotein(a) both as catching and detecting antibodies. The assay measures both free apolipoprotein(a) and apolipoprotein(a) contained in the lipoprotein(a) particle with equal affinity, i.e. the assay measures total apolipoprotein(a). So, there is no reason to think that the apolipoprotein(a) measurements are confounded by a decrease in apolipoprotein B100. In addition, we also found down regulation of apolipoprotein(a) mRNA expression, indicating regulation at the (post-) transcriptional expression.

Riemens and Dullaart refer to a paper by Malmström et al., who report that the VLDL1 apoB synthesis in humans is decreased by 51 % during a period of 8.5 h of hyperinsulinaemia [9]. In that paper the synthesis of apolipoprotein(a) was not

measured and to our knowledge this has not been done yet during hyperinsulinaemia. Because of the half-life of lipoprotein(a), being 2 to 3 days [10], it is not possible to observe statistically significant changes in plasma lipoprotein(a) concentrations during such a short period.

Yours sincerely,
H. M. G. Princen

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Inefficient analysis of twin data: is there an association between diabetes and birth weight?

Dear Sir,

Poulsen et al. [1] examine if there exists a relation between birth weight and Type II (non-insulin-dependent) diabetes mellitus, not due to factors such as maternal height, gestational

age and sex. To be able to control these factors the study was based on twin pairs of the same sex. Unfortunately, the authors did not analyse their data with the most powerful statistical methods and due to this, possible important associations may have been missed. In one of the analyses the twins were divided into two groups, one group (A) containing twins with the heavier birth weight from each pair and one group (B) containing twins with the lighter birth weight (Table 2 p.443). For each independent variable, X_i , The Wilcoxon signed rank test for paired data was calculated. A significant result indicates an association between birth weight and variable X_i .

A more powerful method than the pairwise test described above is to use the correlation between intra-pair differences.

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By using the Wilcoxon test only the sign of the difference in birth weight is taken into account, i.e. twin pairs that differ 1 gram in birth weight are not differentiated from twin pairs who differ many hundreds of grams in birth weight. By using a correlation test, the size of the difference in birth weight is, however, also considered and hence the analysis becomes more powerful (i.e. increasing the chances of detecting an association).

Another disadvantage with the methods used by Poulsen et al. is the need to be able to separate the twins with respect to their birth weight. If the birth weight of the twins within a pair is equal, the data for these twins cannot be used. Therefore, in some of the analyses, Poulsen et al. had to drop 23 of their 109 twin pairs because of similar birth weights within pairs. This problem does not arise in a 'correct' analysis of the data.

To examine if Type II diabetic twins have a statistically significant lower birth weight than non-diabetic twins a correct analysis using the Wilcoxon test based on intra-pair differences (i.e. birth weight of the Type II diabetic twin minus birth weight of the non-diabetic co-twin) was carried out. Unfortunately, the method used to obtain intra-pair differences by always taking the Type II diabetic twin minus the non-diabetic twin also seems to have been used when calculating the correlation between duration of diabetes and birth weight.

When calculating the intra-pair differences, in duration of diabetes and birth weight, to be used in the correlation analysis, it is not necessary to be consequent in the meaning of always taking the Type II diabetic minus the non-diabetic twin or vice versa. It is correct and even more powerful to be inconsequent, i.e. taking the non-diabetic twin minus the Type II diabetic twin for one twin pair and vice versa for another twin pair. Which approach to be applied to which twin pair is decided by randomisation.

By always taking the Type II diabetic minus the non-diabetic twin we will only get positive differences in duration of diabetes. Therefore, the range of possible differences is restricted to only positive values. If we are inconsequent when calculating the intra-pair differences, we can obtain both positive and negative values and the range will be wider. This is important because the correlation coefficient is dependent on the variability in the sample. The larger the variability in the sample the stronger the correlation.

Hence, the procedure used by the authors to calculate intra-pair differences reduced the probability to find a significant relation.

Another approach to examine the relation between duration of diabetes and birth weight is to calculate the intraclass correlation [2, p.273]. In this analysis each twin pair is used twice. Once taking the Type II diabetic twin minus the non-diabetic twin and once taking the non-diabetic twin minus the Type II diabetic twin. A third approach is to analyse the data by means of regression analysis [3]. Observe that the randomisation method described above is not suitable for small samples. For small samples the intra-class and the regression method is more suitable.

Our comments do not invalidate Poulsen et al.'s results, but stronger associations and even more interesting results could have been found if a better statistical analysis of the data had been done. How to analyse twin data in the best possible way is far from trivial. Poulsen et al. are not the first researchers using inefficient methods in the analysis of twin data [4, 5].

Yours sincerely,
J. Bring, L. Wernroth

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Evidence for an association between diabetes and birth weight still exists

Dear Sir,

We thank Bring et al. for their comments. We are pleased that our paper published in April 1997 continues to be of interest [1]. In our study we report the finding of lower birth weights in both monozygotic and dizygotic twins with Type II (non-insulin-dependent) diabetes mellitus compared with their non-diabetic co-twins. These results indicate an association between low birth weight and Type II diabetes mellitus independent of factors such as gestational age, maternal height, birth order and sex. The finding of lower birth weights in monozygotic twins with Type II diabetes compared with their genetically

identical non-diabetic co-twins, furthermore, eliminates the possibility that the association can be due to a genotype causing both Type II diabetes and low birth weight.

In addition to the paired-twin approach (Type II diabetic twin vs non-diabetic twin) we carried out the non-parametric Wilcoxon test for paired data in the comparison of the group of twins with the heavier birth weight and the group of co-twins with the lighter birth weight to detect any differences. Furthermore, we did Spearman correlation analysis between birth weight and other variables.

Bring et al. suggest that a more powerful analysis of our data would be the correlation between intra-pair differences. We *did* perform these analyses (i.e. the "correct" analysis of data). The only significant correlation between intra-pair differences was the correlation between difference in birth weight and difference in 120-min post OGTT plasma glucose ($r = -0.26$, $p < 0.01$). This is mentioned on page 443, paragraph 3. We did not believe that the non-significant correlations between intra-pair differences added to the scientific content and we, subsequently, chose not to show the data.

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