4. Meirhaeghe A, Amoyel P, Helbecque N et al (2000) An uncoupling protein 3 gene polymorphism associated with a lower risk of developing type 2 diabetes and with atherogenic lipid profile in a French cohort. Diabetologia 43: 1424–1428
5. Otabe S, Clement K, Dina C et al (2000) A genetic variation in the 5'flanking region of the *UCP3* gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 43: 245–249
6. Cassell PG, Saker PJ, Huxtable SJ et al (2000) Evidence that single nucleotide polymophism in the uncoupling protein 3 (UCP3) gene influences fat distribution in women of European and Asian origin. Diabetologia 43: 1558–1564
7. Dalgaard LT, Sørensen TIA, Drivsholm T, Borch-Johnsen K, Andersen T, Hansen T, Pedersen O (2001) A prevalent polymorphism in the promoter of the UCP3 gene and its relationship to body mass index and long term body weight change in the Danish population. J Clin Endocrinol Metab 86: 1398–1402
8. Cassell PG, Neverova M, Janmohamed S et al. (1999) An uncoupling protein 2 gene variant is associated with a raised body mass index but not type II diabetes. Diabetologia 42: 688–692

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Observation(s)

Human arterial smooth muscle cells rapidly deplete cell culture media of glucose

To the Editor: Arterial smooth muscle cells contribute to the progression of lesions of atherosclerosis and cardiovascular disease. We have recently shown that accumulation and proliferation of arterial smooth muscle cells in atherosclerotic lesions are markedly stimulated by diabetes in a new porcine model of diabetes-accelerated atherosclerosis [1]. Thus, increased understanding of arterial smooth muscle biology is important for studies on cardiovascular complications of diabetes. Cultured arterial smooth muscle cells provide a useful model that is frequently used for studies in this area.

The rate of glucose consumption varies widely between different cell types and tissues. In this study, we used non-transformed diploid smooth muscle cells isolated by an explant method [1] from the human aorta to determine glucose consumption rates in these cells. Glucose consumption in cultured cells (passage 3–9) was measured as loss of glucose from tissue culture media, using a standard enzymatic method (Sigma, St. Louis, Mo., USA). The rate of glucose consumption was linear for several days as long as glucose concentrations were higher than 2 mmol/l (data not shown). The results show that glucose consumption in proliferating human aortic smooth muscle cells incubated in DMEM containing 10% FCS and 5.6 mmol/l glucose is high compared to that of proliferating lymphocytes [2] and many other cell types. Glucose consumption is; 0.54 ± 0.02 pmoles glucose $\cdot h^{-1} \cdot cell^{-1}$ (means ± SD, $n = 8$), 0.62 ± 0.06 ($n = 6$) and 0.63 ± 0.03 pmoles glucose $\cdot h^{-1} \cdot cell^{-1}$ ($n = 3$), respectively, in human smooth muscle cells isolated from three donors. n represents the number of plates analysed from each donor in one representative experiment. The high rate of glucose consumption in arterial smooth muscle is similar to that of malignant cells that are known to exhibit about a tenfold increase in glucose uptake and high glycolysis under aerobic conditions [3, 4]. Of interest, arterial smooth muscle also has a high rate of glycolysis under aerobic conditions [1, 5]. In this respect normal human arterial smooth muscle cells in culture resemble tumour cells and a few other cell types, e. g. proliferating lymphocytes [2] and thymocytes [6]. It is possible that the increased energy required in cells with a high proliferative capacity is supplied primarily by glycolysis rather than by oxidative glucose breakdown. In this context, it has been suggested that glycolysis, although highly unfavourable for the cell in terms of ATP production, could serve as a protective strategy to minimize oxidative stress [6].

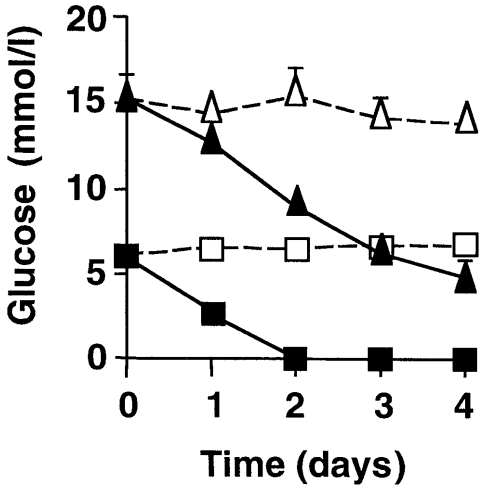

Fig. 1. Human arterial smooth muscle cells rapidly deplete cell culture media of glucose. Arterial smooth muscle cells were isolated from the human thoracic aorta and maintained in culture for eight passages. Smooth muscle cells (217 887 ± 3372 cells/well in 24-well plates) were incubated in DMEM (1 ml/well) containing 5.6 mmol/l glucose (*squares*) or 15 mmol/l glucose (*triangles*) and 10% FCS for 4 days (*closed symbols*). As controls, media were incubated under the same conditions in the absence of cells (*open symbols*). Glucose concentrations in the media were measured using a kit (Trinder) from Sigma (St. Louis, Mo., USA) as described previously [1]. The results are expressed as means ± SD ($n = 3$). Similar results were obtained by using smooth muscle cells maintained in culture for passages 3 to 8.

The high rate of glucose consumption in human arterial smooth muscle cells has implications for studies of effects of different glucose concentrations in these cells. During a relatively short period of time, cell cultures kept in 24-well plates in the presence of 10% FCS (1 ml medium per well) rapidly deplete the medium of glucose unless the medium is replaced frequently. A medium that contains 5.6 mmol/l glucose at the beginning of the study contains only 2.7 ± 0.13 mmol/l glucose after 1 day of incubation and is completely devoid of glucose after 2 days (Fig. 1). Human arterial smooth muscle cells do

not show reduced glucose consumption when exposed to increased glucose concentrations for up to 6 days [1], in contrast to rat and bovine arterial smooth muscle cells exposed to high glucose concentrations for 24 h [7–8]. Accordingly, human arterial smooth muscle cells incubated in the presence of 15 mmol/l glucose reduce glucose concentrations to near normal (4.8 ± 0.97 mmol/l glucose) in 3 days (Fig. 1).

In conclusion, our observations show that normal human aortic smooth muscle cells in culture consume large amounts of glucose and, as a result, these cells rapidly deplete cell culture media of glucose. The results further indicate the advantage of monitoring cell glucose consumption in studies that aim to compare the effects of normal compared with high glucose concentrations on different processes in human arterial smooth muscle cells and other types of cells that could share similar characteristics.

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References

1. Suzuki LA, Poot M, Gerrity RG, Bornfeldt KE (2001) Diabetes accelerates smooth muscle cell accumulation in lesions of atherosclerosis: Lack of direct growth-promoting effects of high glucose levels. Diabetes 50: 851–860
2. Wang T, Marquardt C, Foker J (1976) Aerobic glycolysis during lymphocyte proliferation. Nature 261: 702–705
3. Skøyum R, Eide K, Berg K, Rofstad EK (1997) Energy metabolism in human melanoma cells under hypoxic and acidic conditions in vitro. Br J Cancer 76: 421–426
4. Dang CV, Semenza GL (1999) Oncogenic alterations of metabolism. Trends Brochem Sci 24: 68–72
5. Paul RJ, Bauer M, Pease W (1979) Vascular smooth muscle: aerobic glycolysis linked to sodium and potassium transport processes. Science 206: 1414–1416
6. Brand KA, Hermfisse U (1997) Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB J 11: 388–395
7. Kaiser N, Sasson S, Feener EP et al. (1993) Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. Diabetes 42: 80–89
8. Howard RL (1996) Down-regulation of glucose transport by elevated extracellular glucose concentration in cultured rat aortic smooth muscle cells does not normalize intracellular glucose concentrations. J Lab Clin Med 127: 504–515

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Observation(s)

Increased hospitalization with longer distance from treatment centre in diabetic paediatric patients in Germany

To the Editor: Several factors are believed to determine the effectiveness of diabetes treatment in young people, including the structure of diabetes care [1–4]. It is not clear whether a few large centres, offering highly specialized care but resulting in longer patient travel distances, or a higher number of smaller patient-near centres are more effective. An important indicator of quality of care is hospitalization because of its high individual as well as social cost [5]. We therefore investigated a possible effect of the distance between patients' residence and their treatment diabetes centre on hospitalization in paediatric diabetic patients in Germany.

Based on a prospective computer-based documentation programme, we ascertained hospital admissions and days in 1999 after diabetes onset for each patient under 20 years of age in 89 paediatric departments belonging to a Working Group on Quality Management in Childhood Diabetes [6], as well as sex, age, diabetes duration, metabolic control (HbA_{1c}: SDS-score < 3, 3 to < 5, ≥ 5), occurrence of severe hypoglycaemia (yes/no) and the distance between home and clinic (derived from areas' postal codes and corresponding centrally defined geographical coordinates (Gauss-Krüger): < 20, 20 to < 50, ≥ 50 km). Adjusted relative risks (RR's, 95 %-CI) were estimated using multivariate negative binomial regression models.

A total of 8493 patients (52 % male; mean age 12.3 ± 4.2; mean diabetes duration 4.7 ± 4.2 years; 7455 years of observation time in 1999) were included in the study. The estimated hospitalization incidence and the number of hospital days (per person-year, 95 %-CI) were 0.33 (0.31–0.34) and 2.21 (2.18–2.24), respectively. Hospitalization incidence was significantly higher in girls than in boys and in pubertal subjects aged 10–14 years with diabetes duration of more than 1 year compared with subjects with diabetes duration of less than 1 year. Hospital incidence and days were significantly higher in subjects with poor metabolic control or occurrence of hypoglycaemia (Table 1). Independently from these clinical variables, hospitalization was higher when the distance to be travelled by the patient was more than 50 km compared with under 20 km (Table 1). When the treatment centre was included in the model, an association between hospitalization and centre was found ($p < 0.001$). The association between distance and hospitalization was weaker (hospital incidence: RR 1.14, 0.97–1.34, $p = 0.099$; hospital days: RR 1.37, 1.03–1.82, $p = 0.028$). The magnitude of the association of the other variables did not substantially change (data not shown).

In conclusion, a long distance between a patients' place of residence and their treatment centre is associated with increased hospitalization in diabetic children and adolescents in Germany, in particular, with an increased number of hospital