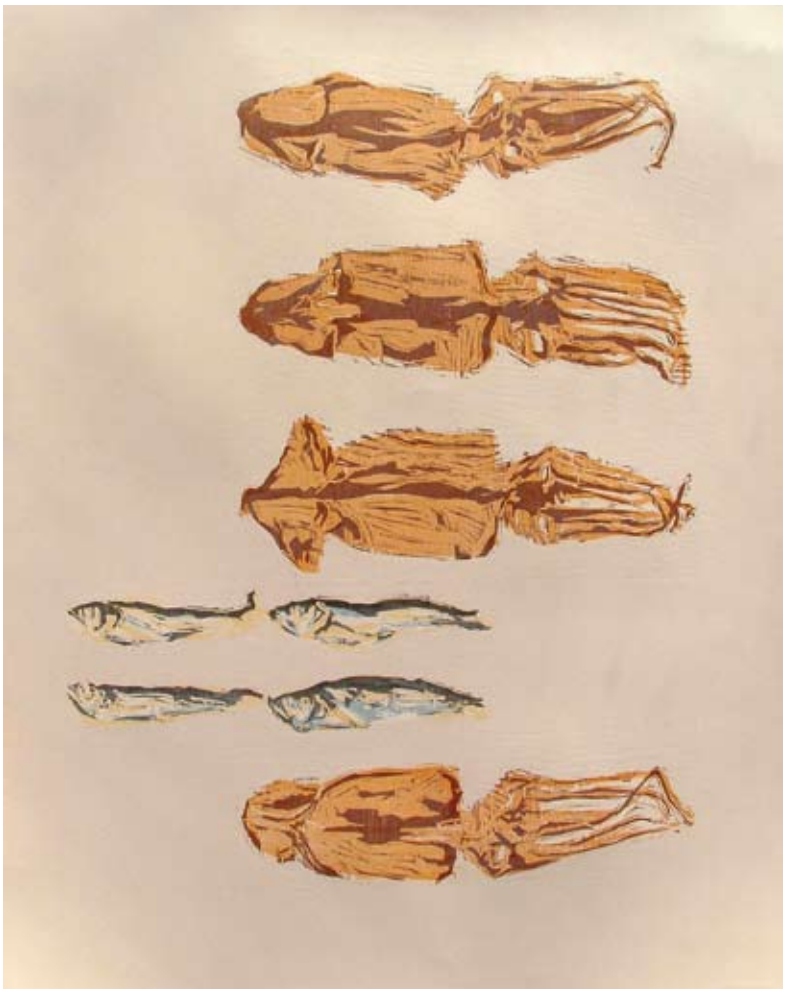
Netherlands
The Journal of Medicine
PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



September 2006, Vol. 64, No. 8, ISSN 0300-2977

VAN ZUIDEN COMMUNICATIONS

Netherlands
The Journal of Medicine
PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE

MISSION STATEMENT

The mission of the journal is to serve the need of the internist to practice up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on speciality training and medical education, book reviews and correspondence.

EDITORIAL INFORMATION

Editorial office 'The Netherlands Journal of Medicine'
Geeralien Derksen-Willemsen
Radboud University Nijmegen Medical Centre
Department of General Internal Medicine 463
PO Box 9101, 6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59
Fax: +31 (0)24-354 17 34
E-mail: g.derksen@aig.umcn.nl
http://mc.manuscriptcentral.com/nethjmed

CITED IN

Biosis database; embase/excerpta medica; index medicus (medline) science citation index, science citation index expanded, isi alerting services, medical documentation services, current contents/clinical medicine, PubMed.

VAN ZUIDEN communications

Contents

Cover
Collage by Manuela Jalimsing. For details about the artist, her work and how to order see elsewhere in this journal.

Subscriptions
General information
An annual subscription to The Netherlands Journal of Medicine (ISSN 0300-2977) consists of 11 issues. Issues within Europe are sent by standard mail and outside Europe by air delivery. Cancellations should be made, in writing, at least two months before the end of the year.

Subscription fee
The annual subscription fee within Europe is € 650, for the USA € 665 and for the rest of the world € 765. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Payment method
Please make your check payable to Van Zuiden Communications B.V., PO Box 2122, 2400 CC Alphen aan den Rijn, the Netherlands or you can transfer the fee to ING Bank, account number 67.89.10.872, Castellumstraat 1, Alphen aan den Rijn, the Netherlands, swift-code: ING BNL 2A. Do not forget to mention the complete delivery address of the Journal.

Claims
Claims for missing issues should be made within two months of the date of dispatch. Missing issues will be mailed without charge. Issues claimed beyond the two-month limit must be prepaid at back copy rates.

Orders, preprints, advertising, changes in address, author or general enquiries
Please contact the publisher.

Van Zuiden Communications B.V.
PO Box 2122
2400 CC Alphen aan den Rijn
The Netherlands
Tel.: +31 (0)172-47 61 91
Fax: +31 (0)172-47 18 82
E-mail: njm@zuidencomm.nl
Internet: www.njm-online.nl

Netherlands
The Journal of Medicine

EDITORIAL

Outcome of coronary revascularisation in diabetes

P. Smits

Departments of Pharmacology and Toxicology, and General Internal Medicine, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands

In this issue, Timmer *et al*. present interesting data on the outcome of coronary revascularisation in diabetic patients in the Netherlands.[1] Based on the recommendation of an expert panel, they assigned all enrolled patients to one of three treatment options: coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI) or pharmacotherapy without revascularisation. In total 872 patients were analysed, 107 of whom had diabetes. The investigators concluded that patients with diabetes experience worse outcomes compared with nondiabetic subjects. Interestingly, the increased long-term mortality in diabetes held true for the PCI subgroup (41 *vs* 24%) as well as for the pharmacotherapy subgroup (65 *vs* 31%), but not for the CABG-treated patients. Long-term mortality after CABG appeared to be exactly the same in diabetic *vs* nondiabetic subjects (24 *vs* 24%). So, the overall conclusions of the authors were 1) long-term mortality of revascularisation procedures is relatively high in diabetes, and 2) total revascularisation by CABG may be superior to PCI or to a conservative pharmacological approach in diabetes.

The first conclusion of Timmer *et al*., a higher long-term mortality after coronary revascularisation in patients with diabetes *vs* nondiabetics, is a well-known phenomenon.[2] Further analysis of this increased cardiovascular mortality shows that this is at least based on three independent mechanisms.

- Type 2 diabetes accounts for up to 95% of all cases with diabetes. Type 2 diabetes often involves the combination of classical risk factors such as hypertension and dyslipidaemia resulting in a relatively advanced state of atherosclerosis in this particular group of patients.[2] Timmer *et al*. did not discriminate between type 1 and type 2 diabetes,[1] but their data clearly show a higher incidence of risk factors in the diabetic patients, suggesting a predominance of type 2 diabetes in their group. Accordingly, the signs of macrovascular disease were much more frequent in the diabetes group as compared with the nondiabetic patients.
- Apart from the concurrence of risk factors in type 2 diabetes, the disease itself appears to be an important independent risk factor for cardiovascular events. Apparently, the atherosclerotic process in diabetic patients has particular features that contribute to a worse prognosis and a worse response to revascularisation. The majority of mechanistic studies point towards a toxic role for high glucose in the vascular complications.[3] Pathophysiological mechanisms such as endothelial dysfunction, increased platelet aggregation, inflammation and advanced glycosylation end products seem to be crucial in glucose-accelerated atherogenesis.[3] These mechanisms are also relevant for type 1 diabetes. In the DCCT trial, intensive glucose lowering was associated with a significant decrease in carotid artery intima-media thickness, and reduced cardiovascular disease by 42%.[4] Although the progression of atherosclerosis in diabetes is often impressive, there does not seem to be a specific feature that enables us to distinguish the diabetic atherosclerotic plaque from a nondiabetic lesion.
- Vascular disease ultimately results in cardiovascular events. The outcome of such an event is not only dependent on the severity of the atherothrombotic process, but is also determined by mechanisms that modulate ischaemia-reperfusion injury. Animal experiments have convincingly shown that a certain vascular obstruction of a coronary artery results in a larger myocardial infarction under hyperglycaemic conditions than under normoglycaemic conditions.[5] Apparently, the diabetic or hyperglycaemic state inhibits endogenous cardioprotective mechanisms. One of the most important protective mechanisms against ischaemia is the well-described phenomenon of 'ischaemic preconditioning'.[6] Ischaemic preconditioning is defined as an increased tolerance to ischaemia and reperfusion induced by a previous sublethal period of ischaemia. Other than timely reperfusion, ischaemic preconditioning is the most powerful mechanism for

limiting infarct size. The myocardial ATP-dependent potassium channel (K_{ATP} channel) plays a crucial role in the cellular signalling of ischaemic preconditioning.[6] Recent research has shown that diabetes attenuates K_{ATP} channel function,[7] thereby explaining reduced ischaemic preconditioning in these patients.[8]

It is interesting to realise that all three aforementioned mechanisms may have been modulated by the use of prescribed drugs in the study of Timmer *et al.* For example, blood pressure and cholesterol-lowering drugs would certainly reduce the impact of the concurring risk factors in type 2 diabetes. From a theoretical point of view, blood glucose lowering drugs are expected to reduce glucose-accelerated atherogenesis, and the positive clinical data of the biguanide derivative metformin on cardiovascular complications may be compatible with this view.[9] Interestingly, blood glucose lowering drugs may also affect ischaemic preconditioning. Sulphonylurea derivatives, and in particular glibenclamide, have been shown to block the myocardial K_{ATP} channel, thereby resulting in a negative effect on ischaemia-reperfusion injury in the human *in vivo* setting.[10-12] Indeed, the use of sulphonylurea drugs is associated with an increased mortality in patients with diabetes mellitus after direct angioplasty following acute myocardial infarction.[13] In contrast, insulin, metformin and thiazolidinedione derivatives appear to limit ischaemia-reperfusion injury in experimental conditions.[14-16] Unfortunately, the study by Timmer *et al.*[1] did not analyse the different pharmacotherapeutic regimens of the included patients. As such, the impact of the aforementioned drug-induced mechanisms cannot be evaluated in their set of data.

The second conclusion of Timmer *et al.* refers to the superiority of CABG *vs* PCI. In a recent article, Flaherty and Davidson reviewed the outcome of subgroups of diabetic patients in six randomised clinical trials comparing CABG with PCI in a total of 950 patients.[2] This overview confirms the superiority of CABG *vs* PCI as far as balloon-only PCI is concerned. However, the data do not support a superiority of CABG *vs* stent-assisted PCI. It seems important to realise that the use of drug-eluting stents has led to dramatic reductions in restenosis in diabetes. Finally, the use of glycoprotein IIb/IIIa antagonists has improved the outcome of PCI in diabetes. As such, the conclusion on the superiority of CABG *vs* PCI may not hold for current strategies with drug-eluting stents and with new pharmacological agents to inhibit coagulation.

In conclusion, studies have shown that the outcome of coronary revascularisation, in particular of balloon-only PCI, is relatively poor in patients with diabetes. However, a thorough analysis of the respective factors that are considered to contribute to this poor outcome can help to optimise the chance of long-term survival after coronary revascularisation.

REFERENCES

1. Timmer JR, Breeman A, Ottervanger JP, de Kluiver EP, Boonstra PW, Zijlstra F. Long-term clinical outcome of patients with diabetes proposed for coronary revasularisation. Neth J Med 2006;64:296-301.
2. Flaherty JD, Davidson CJ. Diabetes and coronary revascularization. JAMA 2006;293;1501-8.
3. Goldberg IJ, Dansky HM. Diabetic vascular disease. An experimental objective. Arterioscler Thromb Vasc Biol 2006;26:1693-701.
4. Natham DM, Cleary PA, Backlund JY, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643-53.
5. Hearse DJ, Stewart DA, Chain EB. Diabetes and the survival and recovery of the anoxic myocardium. J Mol Cell Cardiol 1975;7:397-415.
6. Riksen NP, Smits P, Rongen GA. Ischaemic preconditioning: from molecular characterisation to clinical application--part II. Neth J Med 2004;62:409-23.
7. Bisseling T, Bisseling TM, Versteegen MG, et al. Impaired K_{ATP} channel function in the fetoplacental circulation of patients with type 1 diabetes mellitus. Am J Obstet Gynecol 2005;192:973-9.
8. Riksen NP, Smits P, Rongen GA. Ischaemic preconditioning: from molecular characterisation to clinical application--part I. Neth J Med 2004;62:353-63.
9. UKPDS. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-65.
10. Bijlstra PJ, den Arend JA, Lutterman JA, Russel FG, Thien T, Smits P. Blockade of vascular ATP-sensitive potassium channels reduces the vasodilator response to ischaemia in humans. Diabetologia 1996;39:1562-8.
11. Broadhead MW, Kharbanda RK, Peters MJ, MacAllister RJ. K_{ATP} channel activation induces ischemic preconditioning of the endothelium in humans in vivo. Circulation 2004;110:2077-82.
12. Lee TM, Chou TF. Impairment of myocardial protection in type 2 diabetic patients. J Clin Endocrinol Metab 2003;88:531-7.
13. Garrat KN, Brady PA, Hassinger NL, Grill DE, Terzic A, Holmes DR. Sulfonylurea drugs increase early mortality in patients with diabetes mellitus after direct angioplasty for acute myocardial infarction. J Am Coll Cardiol 1999;33:119-24.
14. Legtenberg RJ, Houston RJ, Oeseburg B, Smits P. Metformin improves cardiac functional recovery after ischemia in rats. Horm Metab Res 2002;34:182-5.
15. Legtenberg RJ, Houston RJ, Oeseburg B, Smits P. Physiological insulin concentrations protect against ischemia-induced loss of cardiac function in rats. Comp Biochem Physiol A Mol Integr Physiol 2002;132:161-7.
16. Khandoudi N, Delerive P, Berrebi-Bertrand I, Buckingham RE, Staels B, Bril A. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma, inhibits the Jun NH(2)-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury. Diabetes 2002;51:1507-14.

EDITORIAL

Download hit list for articles published in the Netherlands Journal of Medicine in 2005

Editorial Board, The Netherlands Journal of Medicine

Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 04 59, fax: +31 (0)24-354 17 34, e-mail: g.derksen@aig.umcn.nl

The Netherlands Journal of Medicine is on the move. Slowly, we have entered the electronic age. First, we opened up our windows and made the complete content of the Journal available to everyone who is interested.[1] All issues from January 2002 onwards are available online at www.njmonline.nl and it is possible to access individual articles via NLM's PubMed (www.pubmed.gov). As a further step, we implemented an online submission system, (http://mc.manuscriptcentral.com/nethjmed) in order to streamline the submission process. This has been greatly successful as the number of submissions has increased sharply. For prospective authors this means that competition will be stiffer and that it will be harder to get into the Journal. On the other hand, this will increase the quality of the content of the Journal. On balance we think that this is a positive development, which will benefit the readers of the Journal.

We, as Editorial Board, are also curious whether and to what extent our readers access the online content of the Journal. Therefore, we analysed the number of downloads for articles that were published in 2005. We believe that our analysis serves several goals. First, it informs us which types of papers (i.e. reviews, case reports) are the most popular and it also gauges which subject areas enjoy popularity among readers. This might influence the path we are taking as Editorial Board. Next, it has been suggested that the number of downloads correlates with the number of citations in subsequent years.[2] As such, the number of downloads is a potentially useful measure of the scientific value of a paper. Also, download requests can be regarded as a highly dynamic measure as it is a direct reflection of immediate research interest rather than citation measurement which only comes to light years after publication of the article.[3] Lastly, everybody likes a pat on the back and the list serves as a tribute to those authors who made it to the list. Our analysis has some inherent limitations. The analysis of our user log file is a rather crude measure as we cannot exclude counts that arise from multiple accesses to the same page by the same person. Furthermore, hits by search machines such as Google generate counts that have not been excluded. Lastly, page views alone may not be an accurate measure of user interest or user perceived quality and usefulness of accessed pages.

Table 1 depicts the first ten most accessed articles. We only adopted the open access model in August 2005 and the analysing software came into place in November 2005,

Table 1. *Top 10 of downloaded articles accessed between 22 November 2005 and 10 August 2006*

Rank	Title	Reference	Hits
1	Psoas abscess: report of a series and review of the literature	4	1067
2	Mediastinal mass	5	473
3	ANCA seropositivity in HIV: a serological pitfall	6	407
4	Establishment of reference values for endocrine tests. Part IV: Adrenal insufficiency	7	402
5	A severe (type II) hepatopulmonary syndrome in a patient with idiopathic portal hypertension and treatment with paroxetine	8	402
6	Idiopathic focal segmental glomerulosclerosis: a favourable prognosis in untreated patients?	9	382
7	Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature	10	371
8	Reverse epidemiology of blood pressure in dialysis patients: implications for treatment?	11	367
9	*Helicobacter pylori*, obesity and gastro-oesophageal reflux disease. Is there a relation? A personal view	12	352
10	Reverse epidemiology: paradoxical observations in haemodialysis patients	13	347

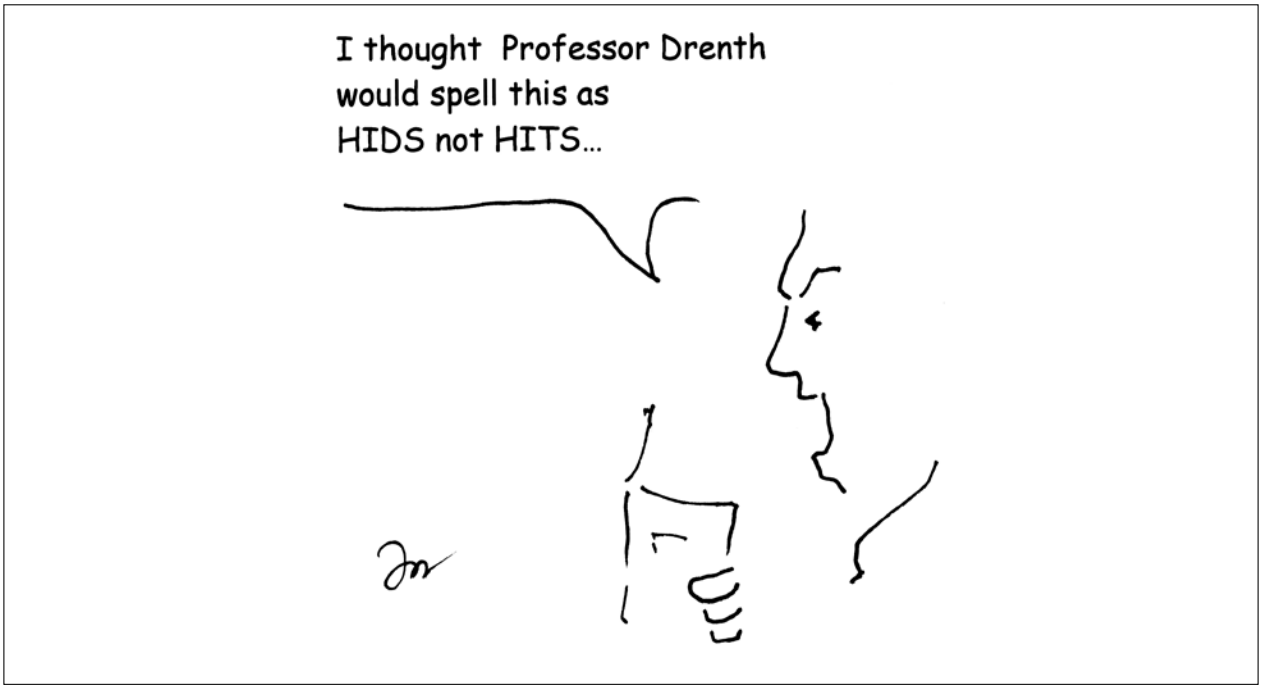
so only downloads after this date are counted. As a consequence articles published in the autumn of 2005 enjoy a head start. There are three reviews, three case reports, two editorials, one original article and, much to our delight, one photo quiz among the most downloaded papers. As you can see it is topped by a case series from Van den Berge *et al*. This article gives clinical details of a series of twelve patients with a psoas abscess and gives an overview on the causes of this disorder.[4] Over the last months this paper was accessed over 1000 times, which is considerable. The photo quiz on mediastinal mass by Monteban-Kooistra and colleagues follows. This paper deals with an atypical presentation of a pancreatic pseudocyst, which happened to be located in the posterior mediastinum.[5] Jansen and colleagues' paper on a false-positive ANCA again hits our headlines.[6] This article won the NJM award for the best case report in 2005, and most deservingly so.

The subjects covered by our highest download list are diverse and range from endocrinology, nephrology to gastroenterology, thus representing the broad interest of our readers.

As a service to our readership and authors, from 1 January 2007 onwards, we will start publishing a monthly list of top downloads from the issue of the preceding month. We hope that you will appreciate this feature in the Journal.

REFERENCES

1. Drenth JP. A watershed for the Netherlands Journal Medicine: open internet access. Neth J Med 2005;63:239-40.
2. Perneger TV. Relation between online "hit counts" and subsequent citations: prospective study of research papers in the BMJ. BMJ 2004;329:546-7.
3. Drenth JP, Smits P, Thien T, Stalenhoef AF. The Netherlands Journal of Medicine's hit list: best cited articles in 2003. Neth J Med 2005;63:418-20.
4. Van den Berge M, de Marie S, Kuipers T, Jansz AR, Bravenboer B. Psoas abscess: report of a series and review of the literature. Neth J Med 2005;63:413-6.
5. Monteban-Kooistra WE, Meertens JH, van der Werf TS. Mediastinal mass. Neth J Med 2005;63:407, 417.
6. Jansen TL, van Houte D, de Vries T, Wolthuis A. ANCA seropositivity in HIV: a serological pitfall. Neth J Med 2005;63:270-4.
7. Endert E, Ouwehand A, Fliers E, Prummel MF, Wiersinga WM. Establishment of reference values for endocrine tests. Part IV: Adrenal insufficiency. Neth J Med 2005;63:435-43.
8. Yilmaz S, Dursum M, Canoruc F, Bayan K, Karabulut A, Akay H. A severe (type II) hepatopulmonary syndrome in a patient with idiopathic portal hypertension and treatment with paroxetine. Neth J Med 2005;63:448-52.
9. Deegens JK, Assmann KJ, Steenbergen EJ, et al. Idiopathic focal segmental glomerulosclerosis: a favourable prognosis in untreated patients? Neth J Med 2005;63:393-8.
10. Halbertsma FJ, Vaneker M, Scheffer GJ, van der Hoeven JG. Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. Neth J Med 2005;63:382-92.
11. Van der Sande FM, Kooman JP, Leunissen KM. Reverse epidemiology of blood pressure in dialysis patients: implications for treatment? Neth J Med 2005;63:373-5.
12. Loffeld RJ. Helicobacter pylori, obesity and gastro-oesophageal reflux disease. Is there a relation? A personal view. Neth J Med 2005;63:344-7.
13. Nurmohamed SA, Nube MJ. Reverse epidemiology: paradoxical observations in haemodialysis patients. Neth J Med 2005;63:376-81.



Editorial Board. Download hit list for articles published in the Netherlands Journal of Medicine in 2005.

Netherlands
The Journal of Medicine

REVIEW

Pathophysiology and management of recurrent hypoglycaemia and hypoglycaemia unawareness in diabetes

B.E. de Galan[1*], B.J.J.W. Schouwenberg[1,2], C.J. Tack[1], P. Smits[1,2 **]

Departments of [1]Medicine and [2]Pharmacology-Toxicology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands, [*]corresponding author: tel.: +31 (0)24-361 88 19/ +31 (0)24-361 32 86, fax: +31 (0)24-354 17 34, e-mail: b.degalan@aig.umcn.nl

ABSTRACT

Iatrogenic hypoglycaemia is a well-known complication of insulin therapy in patients with diabetes mellitus and a limiting factor for glycaemic control. In a setting of endogenous insulin deficiency (type 1 and advanced type 2 diabetes), one episode of hypoglycaemia reduces both counterregulatory hormone responses to and subjective awareness of subsequent hypoglycaemia, thus impairing physiological defences against hypoglycaemia. This phenomenon may lead to a vicious cycle of recurrent hypoglycaemia and glucose counterregulatory failure, of which hypoglycaemia unawareness (i.e. the inability to perceive symptoms of hypoglycaemia) is the clinical representative. The underlying mechanism of hypoglycaemia-induced counterregulatory failure has not yet been disclosed. Patients with clinical hypoglycaemia unawareness are at high risk of severe hypoglycaemia that requires third-party assistance. Management options include avoidance of hypoglycaemic events and optimisation of insulin therapy to limit deterioration of glycaemic control associated with hypoglycaemia avoidance. Several counterregulatory-stimulating agents have been found to improve hypoglycaemic awareness in small clinical trials, but none have been tested in sufficiently large randomised studies to justify their use in daily practice. More research is required to elucidate the pathogenesis of counterregulatory failure and to develop adequate treatment strategies.

KEYWORDS

Diabetes mellitus, glucose counterregulation, hypoglycaemia unawareness, insulin treatment

CASE REPORT

At her three-monthly visit to the outpatient clinic, a 37-year-old woman with type 1 diabetes reported having had a severe hypoglycaemic incident recently. Her five-year-old daughter found her unconscious on the floor and called the neighbours. Only after administration of glucagon intramuscularly did she regain consciousness. She has a 33-year history of diabetes without microvascular complications and is currently being treated with aspart insulin before meals and one injection of glargine at bedtime. Glycaemic control has always been reasonable (but not optimal), with HbA_{1c} values ranging from 7.2 to 8.5%. Hypoglycaemic events are recognised by loss of concentration, diminishing visual acuity, or not at all. Home blood glucose measurements disclose a high number of biochemical hypoglycaemias, the majority of which – the patient admits – were not perceived. Family members often recognise her hypoglycaemic events before she is aware of them herself. She feels insecure, especially when she is alone with her children. She wants to know why hypoglycaemias are so common, why symptoms are no longer perceived, and what led to the severe episode.

Clinical hypoglycaemia rarely occurs in healthy human beings, but it is a fact of life for people with type 1 diabetes mellitus (T1DM) and (advanced) insulin-treated type 2

[**]P. Smits was not involved in the handling and review process of this paper.

diabetes (T2DM). Hypoglycaemia is feared by many patients not only because of the associated physical discomfort, but mainly because of the risk of cognitive function deterioration that may lead to loss of personal control and adequate conscious behaviour, and eventually to coma. Iatrogenic hypoglycaemia has been described ever since the introduction of insulin,[1] and especially those patients attempting to optimise glycaemic control may suffer multiple episodes a week.[2] Numerous studies, including the Diabetes Control and Complications Trial (DCCT)[3] and United Kingdom Prospective Diabetes Study (UKPDS),[4] have established the inverse relationship between HbA_{1c} and risk for hypoglycaemic events. Despite recent advances in insulin treatment, however, iatrogenic hypoglycaemia remains the principal barrier to obtaining true glycaemic control, i.e. blood glucose values that remain within normoglycaemic limits for an indefinite period of time.[5] Thus, microvascular and macrovascular complications of diabetes associated with chronic hyperglycaemia are to a certain extent the consequence of (the risk for) hypoglycaemia. By far not all hypoglycaemic events are recognised, which leads to underreporting of events and increases the risk of hypoglycaemia complicated by coma or epileptic seizures, as the current case exemplifies. In this article, we discuss the pathophysiology of hypoglycaemia in diabetes, its potential to cause harm, and the implications of hypoglycaemia in daily clinical practice, including tactics to reduce its frequency.

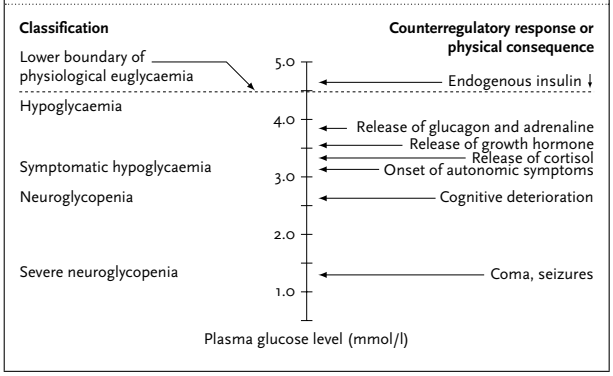
Role of insulin in risk for hypoglycaemia

When discussing potential causes of a hypoglycaemic event with a diabetic patient, it is common to search primarily for the classical risk factors for hypoglycaemia such as missed meals, excess insulin administration, alcohol consumption, and physical activity or sport. However appropriate this may be, many instances of hypoglycaemia are not the result of patient mistakes, but relate to imperfections in therapeutic insulin as opposed to endogenous insulin. In the nondiabetic individual, endogenous insulin release is instantaneous and tailor-made to the amount of carbohydrates that enter the circulation or to any other increase in the blood glucose level. Moreover, after its release by the pancreatic β-cell, insulin first reaches the liver via the portal vein to stimulate hepatic glycogen synthesis and to inhibit hepatic gluconeogenesis. Within the liver, insulin is degraded by approximately 50%,[6,7] so that only half of the insulin released reaches the periphery to stimulate skeletal muscle glucose disposal or to inhibit lipolysis in adipose tissue. With therapeutic insulin, insulin levels are unregulated and do not decrease until the subcutaneous depot is depleted, even though the plasma glucose level may have started to fall (too low). In addition, insulin injected subcutaneously enters the circulation much slower and follows the reverse route, so that elevated insulin levels persist considerably longer. Variations in insulin absorption may explain why a dose of insulin sufficient to maintain normoglycaemia at one time may be too much at other times. All these factors may lead to inappropriate hyperinsulinaemia, i.e. despite corrected hyperglycaemia, hence creating a risk for hypoglycaemia.[8]

Normal glucose counterregulation

In the nondiabetic individual, declining blood glucose levels trigger a characteristic and hierarchically organised sequence of responses (*figure 1*).[9,10] First and foremost, insulin secretion is suppressed when blood glucose levels fall within the physiological range (below 4.5 mmol/l). The resultant reduction in peripheral glucose uptake and increase in hepatic glucose production usually terminates the decline in blood glucose and prevents true hypoglycaemia almost without exception. In addition, the fall in intra-islet insulin appears to have a signalling role for the glucagon response to hypoglycaemia by alleviating its suppressive effect on pancreatic α-cells, thus permitting glucagon release.[11-13] The release of both glucagon and adrenaline is triggered when plasma glucose values fall below ~3.8 mmol/l. They promote hepatic glucose production by stimulation of glycogenolysis and gluconeogenesis. In addition, adrenaline inhibits peripheral glucose uptake, thus contributing to mobilisation of gluconeogenic precursors. Yet adrenaline is normally not critical, provided the glucagon response is intact. Cortisol and growth are released in response to prolonged hypoglycaemia, but have a low significance for acute glucose counterregulation. Plasma glucose values of 3.0 to 3.5 mmol/l trigger central nervous system (CNS) mediated onset of autonomic warning symptoms such as hunger, sweating and palpitations, all of which are fundamental for subjective awareness of hypoglycaemia. These symptoms are aimed to provoke eating behaviour and can be seen as

Figure 1. *Schematic presentation of physiological glycaemic threshold values for counterregulatory responses to and physical consequences of insulin-induced hypoglycaemia*



a last resort before neuroglycopenia develops and cognitive function declines, both of which reflect CNS glucose deprivation (*table 1*). The glucose levels at which these counterregulatory responses to hypoglycaemia occur, also referred to as glycaemic thresholds, are reproducible in healthy subjects, yet they can be altered to higher glucose levels following chronic hyperglycaemia[14] or to lower glucose levels following repeated hypoglycaemia.[15-17] The magnitude of counterregulatory function as a whole tends to decrease with age[18] and is more prominent in men than in (premenopausal) women.[19]

Table 1. *Symptoms of hypoglycaemia*

Autonomic symptoms	Neuroglycopenic symptoms
Sweating	Blurred vision
Tingling	Difficulty speaking
Trembling	Feeling faint
Feeling shaky	Difficulty thinking
Feeling hungry	Confusion
Palpitations	Dizziness
Anxiety	Feeling drowsy
	Irritability

Glucose counterregulation in diabetes

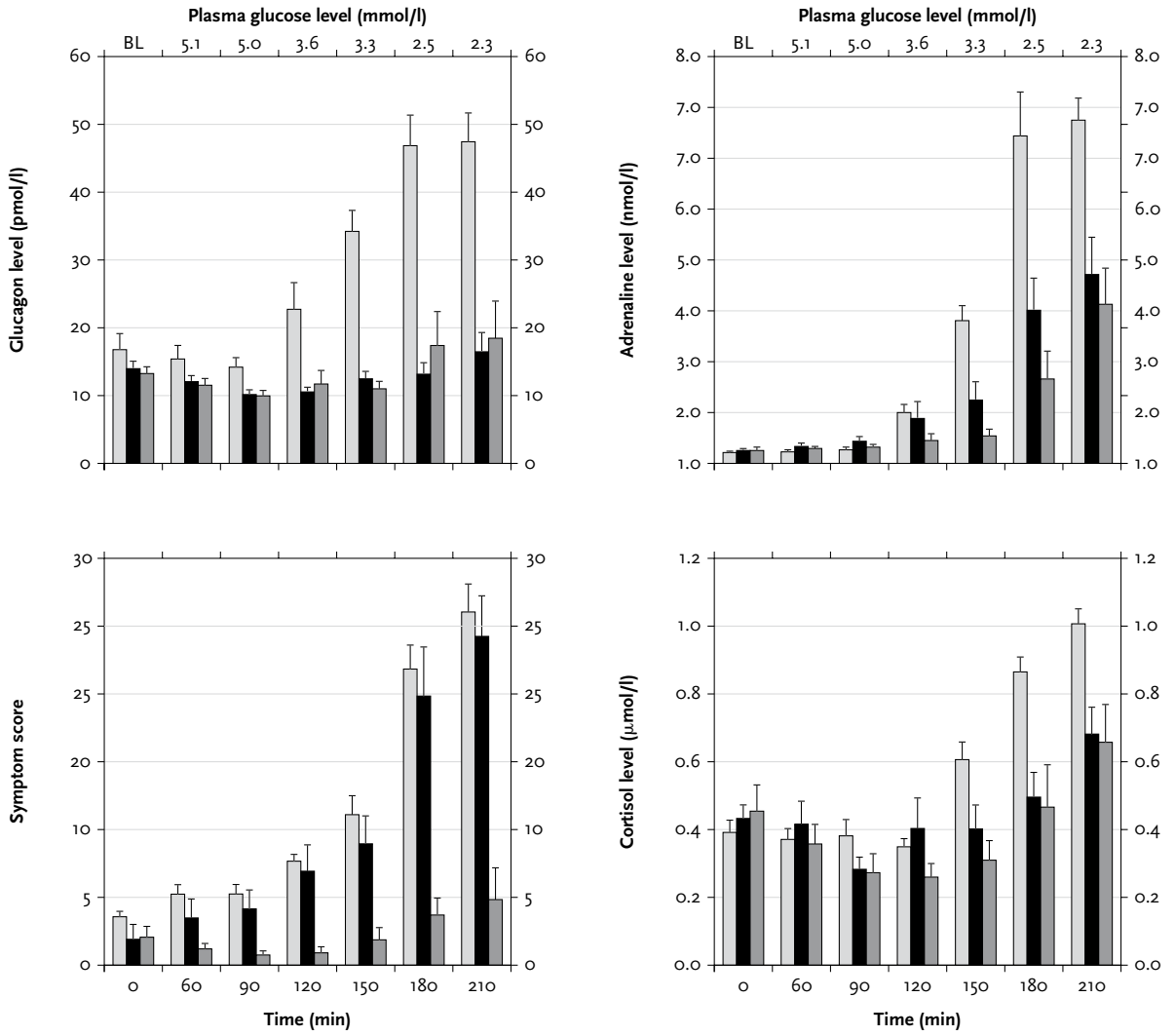
Glucose counterregulation in patients with T1DM is typically impaired. The loss of insulin-producing capacity disrupts the first-line defence against falling blood glucose levels and the consequent lack of paracrine control of the pancreatic α-cell precludes an adequate glucagon response. Therefore, hypoglycaemia usually fails to trigger glucagon responses in TIDM within years after diagnosis. When glucagon responses to hypoglycaemia are deficient, adrenaline and autonomic warning symptoms become critical for the integrity of glucose counterregulation. Iatrogenic hypoglycaemia, however, attenuates the magnitude of adrenaline and autonomic symptom responses to a subsequent hypoglycaemic episode, shifts the glycaemic threshold for these responses to lower levels of glycaemia,[20,21] impairs hypoglycaemic perceptibility clinically,[22] and possibly reduces β-adrenergic sensitivity.[23,24] Any hypoglycaemia, whether mild, asymptomatic,[17] nocturnal,[25] or brief,[26] can provoke this phenomenon. Consequently, a downward vicious cycle of worsening counterregulation and recurrent hypoglycaemia may ultimately lead to hypoglycaemia unawareness. Hypoglycaemia unawareness is defined as onset of neuroglycopenia before the appearance of autonomic warning symptoms, and typified clinically by the inability to perceive hypoglycaemia by symptoms (*figure 2*). Patients with hypoglycaemia unawareness are unable to manifest an adequate behavioural response to avert hypoglycaemia and are at a specifically high risk for severe, disabling hypoglycaemia (e.g. complicated by coma or seizures) that requires external assistance.[27] Various terms are used for the combination of defective hormonal counterregulation and hypoglycaemia unawareness, such as counterregulatory failure, hypoglycaemia-associated autonomic failure (HAAF)[28] and hypoglycaemia unawareness syndrome. Risk factors other than recent antecedent or recurrent hypoglycaemia include good glycaemic control (which by inference is also an index of hypoglycaemic incidence), C-peptide negativity, male sex, and advanced diabetes duration. Diabetic autonomic neuropathy causes many of the counterregulatory defects that are found in patients with hypoglycaemia unawareness.[29-31] However, most patients with hypoglycaemia unawareness have no signs of autonomic neuropathy or microangiopathy. In cross-sectional studies, classical diabetic autonomic neuropathy was not associated to counterregulatory failure or hypoglycaemic incidence.[32,33]

In T2DM, residual β-cell function largely preserves the first-line defence against hypoglycaemia. Consequently, the glucagon response is retained, hypoglycaemic risk is limited and further counterregulatory defects are prevented.[34] Indeed, hypoglycaemic rates are >15-fold lower in orally treated and still approximately sixfold lower in insulin-treated T2DM patients [4] than in patients with T1DM.[3] Insulin resistance and – possibly – increased sensitivity to plasma catecholamines[35] may contribute to prevention of iatrogenic hypoglycaemia and preservation of glucose counterregulation. However, the concept of hypoglycaemia-induced diminution in counterregulatory function also applies to T2DM.[36] Moreover, insulin deficiency in T2DM causes an absent glucagon response to hypoglycaemia[37] and a steep rise in the risk for hypoglycaemia that approaches that of T1DM.[38] T2DM patients who approach the insulin-deficient state are prone to the same counterregulatory defects as patients with T1DM, including hypoglycaemia unawareness.[37] Recent data indicate that 8 to 31% of insulin-treated T2DM patients report having trouble in correctly identifying hypoglycaemic events,[39,40] and that these patients have a ninefold higher risk for severe iatrogenic hypoglycaemia than patients with normal hypoglycaemic awareness.[39]

Pathogenesis of counterregulatory failure

Although the role of antecedent hypoglycaemia in the development of counterregulatory failure is undisputed, the underlying mechanism has yet to be determined. It has been hypothesised that increased cortisol levels during antecedent hypoglycaemia could act as mediator to reduce counterregulatory responses to subsequent hypoglycaemia. This hypothesis was based on the finding

Figure 2. *Responses of glucagon, adrenaline and cortisol to insulin-induced hypoglycaemia in nondiabetic control subjects (light grey bars), T1DM patients with intact hypoglycaemic awareness (black bars) and T1DM patients with hypoglycaemia unawareness (dark grey bars)*[121,122]



Compared with control subjects, both T1DM groups exhibit absent glucagon responses and reduced adrenaline and cortisol responses to hypoglycaemia. In T1DM patients with hypoglycaemia unawareness, the onset of the adrenaline response is shifted to a lower glucose level and hypoglycaemic symptoms are largely absent during hypoglycaemia. BL denotes baseline.

that prior supraphysiological elevation of plasma cortisol was able to mimic some of the – primarily metabolic – effects of antecedent hypoglycaemia[41,42] and that failure to secrete cortisol could prevent the phenomenon.[43] However, prior cortisol elevation did not affect symptomatic awareness of subsequent hypoglycaemia, and lower cortisol levels comparable with those elicited by hypoglycaemia could not reproduce the phenomenon.[44-46] Thus, the cortisol hypothesis provides no explanation for clinical hypoglycaemia unawareness.

Since the CNS plays such a pivotal role in the sensing of hypoglycaemia and the activation of counterregulation, many studies on the pathogenesis of counterregulatory failure have focussed on the brain. In humans and rodents, prolonged (days to weeks) hypoglycaemia was found to increase cerebral glucose uptake,[47,48] possibly mediated through increased expression of cerebral GLUT-1 and GLUT-3 glucose transporters.[49,50] These data led to the suggestion that recurrent hypoglycaemic events preserved or increased brain glucose uptake, thereby shifting hypoglycaemic symptom perception and onset of counterregulatory responses to lower levels of (systemic) hypoglycaemia.[51-53] Observations of increased cerebral glucose content in T1DM patients with near-normal HbA_{1c} values,[54] or with hypoglycaemia unawareness[55] compared with controls seem to support this hypothesis. However, the induction of hypoglycaemia

unawareness was not accompanied by alterations in global blood-to-brain glucose transport when shorter periods of antecedent hypoglycaemia were used, neither in humans[56] nor in animals.[57,58] Moreover, no differences in blood-to-brain glucose transport were found using positron emission tomography between hypoglycaemia aware and hypoglycaemia unaware T1DM patients.[59]

Another possibility is that recent antecedent hypoglycaemia causes alterations in the brain's glucose-sensing neurons in the ventromedial hypothalamus (VMH) that initiate the glucose counterregulatory response (except glucagon). As a result, the onset of counterregulatory responses would then shift to deeper levels of hypoglycaemia. Increased glucokinase activity,[60] K_{ATP} channel closure,[61] decreased AMP-activated protein kinase activity,[62] and reduced insulin signalling[63] have all been suggested as underlying mechanism, but none have been universally established. Finally, hypoglycaemia-induced alteration of brain (glucose) metabolism may be involved in the pathogenesis of counterregulatory defects. Administration of 2-deoxyglucose, a glucose compound that cannot be metabolised, leads to stimulation of glucose counterregulation and hyperglycaemia.[64] Conversely, administration of nonglucose substrates for metabolism, such as β-hydroxybutyrate or lactate, during hypoglycaemia suppress counterregulatory responses.[65-67] Recent data indicate that hypoglycaemia causes twofold higher increases in brain acetate in T1DM patients than in healthy controls[68] without affecting brain energy metabolism,[69] suggesting that increased blood-to-brain transport of alternative metabolic substrates could be the mechanism underlying counterregulatory failure. How hypoglycaemia affects brain glucose metabolism and if alterations therein occur after repeated hypoglycaemia is still unknown. Localised ^{13}C nuclear magnetic resonance (NMR) spectroscopy, provides a novel highly sophisticated tool to study brain glucose metabolism *in vivo*. Under optimal conditions, it has become feasible to use ^{13}C NMR spectroscopy in humans under hypoglycaemic conditions.[70]

Morbidity and mortality of severe hypoglycaemia

Severe decrements in blood glucose levels that interfere with the individual's ability for self-management have the potential to cause significant physical harm. It has been estimated that 2 to 4% of all deaths in T1DM occur under hypoglycaemic conditions.[71] In most instances, death cannot be attributed directly to hypoglycaemia, but relates to the circumstances under which the hypoglycaemic event evolved, e.g. in traffic, during swimming or scuba diving, at heights, et cetera. To avoid hypoglycaemia while driving, it is recommended to test blood glucose before and at regular intervals during driving, to ingest prophylactic carbohydrates when blood glucose is below 5 mmol/l, and to keep an emergency supply of carbohydrates for treatment purposes and to cope with unexpected delays, such as traffic jams.[72]

A direct relation between hypoglycaemia and death has been proposed in the *dead in bed syndrome*, a rare disorder characterised by an unexpected death in a young, previously healthy, tightly controlled T1DM patient, often with a history of recurrent (nocturnal) hypoglycaemia.[73,74] Death in this syndrome is thought to be the result of a fatal ventricular arrhythmia caused by hypoglycaemia-induced lengthening of the QT interval.[75] It is not yet known to what extent hypoglycaemia contributes to mortality in T2DM, yet the fact that hypoglycaemia can induce arrhythmia may be clinically relevant in a population prone to cardiovascular disease (such as T2DM).[76] Moreover, hypoglycaemia as a cause of out-of-hospital mortality may be easily missed in the elderly T2DM population.

It is unclear whether severe hypoglycaemia constitutes a risk factor for persistent loss of cognitive function. There are occasional case reports of serious brain damage induced by severe hypoglycaemia,[77-80] and a recent study reporting altered brain structure after severe hypoglycaemia.[81] In addition, insulin-treated diabetic patients with a history of repeated severe hypoglycaemia were found to perform slightly worse on an IQ test than patients without such a history.[82] Prospective studies[83-85] and a recent cross-sectional study,[86] however, have been unable to establish an association between severe hypoglycaemia and cognitive decline. On the contrary, cognitive dysfunction and structural abnormalities on brain MRI scans appeared to be more prevalent in patients with microvascular complications such as diabetic retinopathy than in those with a history of severe hypoglycaemia.[86] Indeed, evidence is accumulating that loss of cognitive function may result from hyperglycaemia or hyperglycaemia-induced micro- or macroangiopathy, a condition for which the term 'diabetic encephalopathy' has been proposed.[87] As a corollary, cognitive function may benefit more from strict glycaemic control and prevention of hyperglycaemia than from meticulous avoidance of (severe) hypoglycaemia. Studies with extended follow-up, preferably 30 years or longer, are needed to identify whether or not diabetic patients with diabetic encephalopathy are at increased risk of hypoglycaemia-induced brain damage.

Diagnosis of clinical hypoglycaemia unawareness

There are no tests available to definitively establish the presence of hypoglycaemia unawareness or defects in hormonal glucose counterregulation in daily practice. Diagnosis of hypoglycaemia unawareness is subject to clinical judgment, the assessment of which is clinically relevant because of its predictive value for the frequency of severe hypoglycaemic episodes.[39,88] Self-reported failure to perceive hypoglycaemic symptoms is associated with a ninefold higher risk of severe hypoglycaemic events,[88] underscoring patients' capability for reliable self-diagnosis. Clinical signs suggestive of hypoglycaemia unawareness include self-reporting of biochemical

hypoglycaemia unaccompanied by symptoms, loss of autonomic symptoms as initial sign of hypoglycaemia, a (recent) history of severe hypoglycaemia (e.g. coma), or the reporting that lower blood glucose levels are required to elicit symptoms. Nocturnal hypoglycaemias should be specifically addressed as they are more frequent then previously acknowledged and go typically unnoticed.[89] Continuous glucose monitoring (CGMS) may help to detect nocturnal or otherwise asymptomatic hypoglycaemia, but its tendency to overestimate the time spent under hypoglycaemic conditions and its relatively low accuracy during hypoglycaemia should be taken into account.[90,91] It may prove valuable to interview spouses and family members, as they often recognise (the neuroglycopenic symptoms of) hypoglycaemic events before the patient with hypoglycaemia unawareness perceives them. As irritability or even frank aggression may be a consequence of neuroglycopenia,[92] such instances can be disturbing for both patients and their relatives, especially when the patient denies being hypoglycaemic and refuses to take appropriate action. A peculiar observation concerns the expression of odd behaviour by dogs whose owners are in a hypoglycaemic state,[93,94] a phenomenon that might be utilised for hypoglycaemia alerting.[94]

TREATMENT OF HYPOGLYCAEMIA UNAWARENESS AND COUNTERREGULATORY DEFECTS

Table 2 shows all the treatment options for the management of hypoglycaemia unawareness and the counterregulatory defects.

Hypoglycaemic risk reduction

Several studies have found that hypoglycaemia unawareness is reversible, at least in part, when hypoglycaemic events are meticulously avoided. Avoidance of hypoglycaemia for two to three weeks appears sufficient to restore symptomatic awareness of hypoglycaemia, to improve the adrenaline response to hypoglycaemia, to shift the glycaemic thresholds for these responses to higher plasma glucose levels,[95-97] and to normalise β-adrenergic sensitivity.[98] Not all counterregulatory defects respond to this strategy: glucagon responses typically remain unaffected, whereas adrenaline responses improve but usually do not normalise, possibly reflecting more or less permanent loss of adrenaline-releasing capacity.[99] By close monitoring of blood glucose excursions (including at night) and intensifying patient-doctor contacts, it has been reported feasible to limit deterioration of glycaemic control to <1% increase in HbA_{1c}.[100] However, in daily practice the associated worsening of glycaemic control is often much greater, constituting a significant drawback to the strict hypoglycaemia avoidance approach. Therefore, this strategy should be reserved for patients for whom the benefit of avoiding hypoglycaemias clearly outweighs the long-term harm of poorer metabolic control. For example, patients with a history of severe, complicated hypoglycaemia, patients who run the risk that hypoglycaemias result in potentially fatal (traffic) accidents and perhaps patients – or whose relatives – who cannot cope mentally with the burden of recurrent hypoglycaemias.[101] Preferably, tight glycaemic control should be gradually re-introduced once hypoglycaemic awareness has returned.

Table 2. *Treatment options for the management of hypoglycaemia unawareness*

Options	Mechanism	Comment
Reducing hypoglycaemia risk	Avoidance of hypoglycaemia	Two to three weeks is sufficient to improve hypoglycaemia unawareness clinically
Optimising insulin treatment	Idem	Effect on counterregulation depends on effectiveness of hypoglycaemia avoidance
Pharmacological therapy		
• Alanine	Stimulation of glucagon response	Not tested in clinical trials
• β_2-adrenergic agents	Enhancement of adrenaline effect	Not tested in clinical trials
• Methylxanthine derivatives	CNS stimulation	May be efficacious, but emergence of tolerance may limit effect of long-term use
• K_{ATP} channel modulators	Modulation of hypoglycaemia sensing	Not effective in humans, possibly due to inability to cross blood-brain barrier
• Fructose	Idem	Promising, but not tested in clinical trial
Miscellaneous		
• Blood glucose awareness training	Improving accuracy of hypoglycaemia detection	Intensive programme that has only been found effective at the hands of its founders
• High-intensity exercise	Prevention of exercise-induced hypoglycaemia	Single observation in a limited number of subjects

Optimisation of insulin treatment: insulin analogues and CSII

Because of its pivotal role in counterregulatory impairments, any reduction in the rate of iatrogenic hypoglycaemia will automatically support the integrity of counterregulatory function and contribute to prevention of subsequent episodes. Practising hypoglycaemia risk reduction within the boundaries of glycaemic control can be attained by selecting insulin preparations that best mimic the physiological profile of rapid bursts of insulin secretion at mealtimes and stable, peak-less insulin levels between meals and overnight. In general, short-acting insulin analogues, such as lispro or aspart, are associated with a lower rate of hypoglycaemia, especially in the postabsorptive state,[102-104] and preserve counterregulatory function better[105] than regular insulin. Basal insulin is best replaced by a long-acting insulin analogue.[103,106-109] These agents produce a lower peak concentration and have a more predictable pharmacokinetic profile than NPH insulin, which may specifically reduce the risk of nocturnal hypoglycaemia. Finally, continuous subcutaneous insulin infusion (CSII), preferably with a short-acting insulin analogue, is viewed as the best method to match insulin administration to daily fluctuating requirements,[110-113] for which clinical hypoglycaemia unawareness is an accepted indication. Conceptually, use of CGMS may be of adjunctive value to optimise insulin therapy, yet studies addressing this issue are few and have produced conflicting results.[114] An extensive discussion on treatment optimisation, however, is beyond the scope of this review.

Pharmacological management of hypoglycaemia unawareness

Although providing therapeutic insulin in a more physiological fashion can minimise the risk of iatrogenic hypoglycaemia, this alone is often insufficient to normalise hypoglycaemic awareness or hormonal glucose counterregulation. From a pharmacological point of view, several agents have the potential to stimulate or support glucose counterregulation more directly. A number of approaches have been tested.

A first approach is to stimulate hormonal counterregulation directly in order to prevent the occurrence of or promote recovery from hypoglycaemic events. Alanine can enhance the glucagon response to hypoglycaemia and has been found to support recovery from experimental hypoglycaemia[115] and to prevent nocturnal hypoglycaemia somewhat better than a bedtime snack.[116] The remarkable finding of a discernible glucagon response to hypoglycaemia in patients with long-term T1DM in the postprandial period has been attributed to alanine in the meal.[117] Analogously, the β_2-adrenergic agonist terbutaline provides similar clinical effects by enhancing the glucose-stimulating action of adrenaline.[115,116] Theoretically, reduced β-adrenergic sensitivity may limit its usefulness in patients with hypoglycaemia unawareness,[23,24] but recent evidence indicates that responsiveness to β_2-adrenergic agonists is unaltered in these patients.[118] Neither alanine nor terbutaline have been studied in a clinical trial.

A second approach to enhance glucose counterregulation is by CNS stimulation. Several studies have examined the central stimulant effects of the methylxanthine derivatives theophylline and caffeine for their capacity to ameliorate hypoglycaemia unawareness. Both agents have been found to enhance counterregulatory hormone (except glucagon) responses to, the perception of, and recovery from hypoglycaemia in uncomplicated T1DM patients[119,120] and in T1DM patients with hypoglycaemia unawareness.[121] Despite the well-known emergence of tolerance associated with prolonged use of methylxanthines under nonhypoglycaemic conditions, some of the glucose counterregulation enhancing effects are retained during hypoglycaemia.[122] In a clinical setting, three months of caffeine use in a group of nonselected T1DM patients resulted in increased reporting of symptomatic hypoglycaemia and a reduction in biochemical (i.e. asymptomatic) hypoglycaemia, whereas glycaemic control remained unaffected.[123] These promising data need confirmation by larger controlled studies of longer duration before use of caffeine or theophylline can be recommended for the management of hypoglycaemia unawareness.

A final approach is to manipulate cerebral hypoglycaemia sensing, for instance by agents that alter glucokinase activity or K_{ATP} channel opening. Fructose, which may enhance cerebral hypoglycaemia sensing by modulation of glucokinase activity, has been found to stimulate glucagon and adrenaline responses to hypoglycaemia, to stimulate hepatic glucose production and to inhibit peripheral glucose uptake.[124,125] Whether fructose ameliorates hypoglycaemia unawareness and if so, whether the effects of fructose are sustained with long-term use has, however, not been studied so far. In nondiabetic subjects, neither glibenclamide (a K_{ATP} channel blocker) nor diazoxide (a K_{ATP} channel opener) have been found to affect counterregulatory responses to hypoglycaemia,[126] possibly because the agents were unable to cross the blood-brain barrier.

Nonpharmacological interventions

Focusing primarily on the clinical problem of hypoglycaemia unawareness, Cox and co-workers developed what is known as blood glucose awareness training (BGAT), a specific training programme to improve the accuracy of blood glucose level estimation.[127] The BGAT programme involves instruction on (discrete) physical symptoms as internal cues and on the effect of diet, physical exercise, insulin pharmacology, and last blood glucose reading as external cues to allow more adequate assessment of blood glucose level. Although originally developed to improve detection of hyperglycaemia, BGAT has been found to support the accuracy of hypoglycaemia

detection and to decrease the number of asymptomatic hypoglycaemia without compromising glycaemic control,[128] even in the long term.[129] Counterregulatory function may benefit from this reduction in hypoglycaemic incidence – and the detection of falling blood glucose levels at earlier stages – in that a near-normal adrenaline response to insulin-induced hypoglycaemia can be retained.[130] Attempts to translate BGAT into a Dutch version have only been partially successful, as its effect on hypoglycaemia detection was far less evident.[131,132]

Patients who experience hypoglycaemia during or shortly following low- or intermediate-intensity exercise may benefit from a brief period of high-intensity exercise to revert the fall in blood glucose. In seven T1DM patients who exercised for 20 minutes at 40% of maximal capacity, 10 seconds of sprinting on a cycle ergometer was sufficient to stabilise glucose levels and to prevent hypoglycaemia without reductions in insulin use.[133] Interval training, in which low- and high-intensity exercise is alternated, may be equally effective to avoid exercise-induced hypoglycaemia.[134]

CONCLUSION

In insulin-treated diabetic patients, iatrogenic hypoglycaemia is typically the result of the interplay of insulin excess on the one hand and counterregulatory failure, as reflected by defective hormonal counterregulation and hypoglycaemia unawareness, on the other. Although rarely causing direct physical harm, iatrogenic hypoglycaemia constitutes an important burden for patients with T1DM or advanced T2DM, and is the limiting factor in the glycaemic management of diabetes. Use of insulin analogues, both short- and long-acting, and insulin pumps may help to limit hypoglycaemic risk. However, provided that insulin treatment and blood glucose self-monitoring are optimised, improvement of glycaemic control can at some point only be achieved at the expense of increased hypoglycaemic incidence. Conversely, it may prove difficult, if not impossible, to apply hypoglycaemia risk-reducing strategies without compromising glycaemic control to a certain extent. Unless we learn to overcome the imperfections of therapeutic insulin by providing insulin in a much more physiological way (e.g. by glucose-regulated insulin replacement), iatrogenic hypoglycaemia will remain a daily issue for all diabetic patients requiring insulin treatment. More effort should be exercised to understand the pathophysiology of counterregulatory impairments and hypoglycaemia unawareness more thoroughly, in order to develop targeted strategies that support glucose counterregulation and – consequently – reduce hypoglycaemic incidence.

REFERENCES

1. Lawrence RD. Insulin hypoglycaemia. Changes in nervous manifestations. Lancet 1941;ii:602-4.
2. Gross TM, Mastrototaro JJ, Fredrickson LP. Detection of unseen hypoglycemia using continuous glucose monitoring. Diabetologia 2003;43(suppl 1):A5-19.
3. The diabetes control and complications trial research group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 1993;329:977-86.
4. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837-53.
5. Cryer PE. Hypoglycaemia: The limiting factor in the glycaemic management of Type I and Type II Diabetes*. Diabetologia 2002;45:937-48.
6. Eaton RP, Allen RC, Schade DS. Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. J Clin Endocrinol Metab 1983;56:1294-300.
7. Porksen N. The in vivo regulation of pulsatile insulin secretion. Diabetologia 2002;45:3-20.
8. Heller SR. Diabetic hypoglycaemia. Baillieres Best Pract Res Clin Endocrinol Metab 1999;13:279-94.
9. The DCCT Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. Am J Med 1991;90:450-9.
10. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest 1987;79:777-81.
11. Mitrakou A, Ryan C, Veneman T, et al. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. Am J Physiol 1991;260:E67-74.
12. Unger RH. The Berson memorial lecture. Insulin-glucagon relationships in the defense against hypoglycemia. Diabetes 1983;32:575-83.
13. Ter Braak E, Appelman A, Erkelens D, van Haeften T. Glibenclamide decreases glucagon release during mild hypoglycaemia. Diabetologia 1998;41(suppl 1):A68.
14. Banarer S, McGregor VP, Cryer PE. Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. Diabetes 2002;51:958-65.
15. Boyle PJ, Schwartz NS, Shah SD, Clutter WE, Cryer PE. Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. N Engl J Med 1988;318:1487-92.
16. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. Diabetes 1991;40:223-6.
17. Davis MR, Shamoon H. Counterregulatory adaptation to recurrent hypoglycemia in normal humans. J Clin Endocrinol Metab 1991;73:995-1001.
18. Davis SN, Shavers C, Mosqueda Garcia R, Costa F. Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. Diabetes 1997;48:1328-35.
19. Davis SN, Fowler S, Costa F. Hypoglycemic counterregulatory responses differ between men and women with type 1 diabetes. Diabetes 2000;49:65-72.
20. Meneilly GS, Cheung E, Tuokko H. Altered responses to hypoglycemia of healthy elderly people. J Clin Endocrinol Metab 1994;78:1341-8.
21. Mei Q, Mundinger TO, Lernmark A, Taborsky GJ Jr. Early, selective, and marked loss of sympathetic nerves from the islets of BioBreeder diabetic rats. Diabetes 2002;51:2997-3002.

22. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. Diabetes 1988;37:901-7.

23. Dagogo Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. J Clin Invest 1993;91:819-28.

24. De Galan BE, Tack CJ, Willemsen JJ, Sweep CG, Smits P, Lenders JW. Plasma metanephrine levels are decreased in type 1 diabetic patients with a severely impaired epinephrine response to hypoglycemia, indicating reduced adrenomedullary stores of epinephrine. J Clin Endocrinol Metab 2004;89:2057-61.

25. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV. Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. N Engl J Med 1983;308:485-91.

26. Bolli GB, De Feo P, De Cosmo S, et al. A reliable and reproducible test for adequate glucose counterregulation in type I diabetes mellitus. Diabetes 1984;33:732-7.

27. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes Care 1994;17:697-703.

28. Trovik TS, Jaeger R, Jorde R, Sager G. Reduced sensitivity to beta-adrenoceptor stimulation and blockade in insulin dependent diabetic patients with hypoglycaemia unawareness. Br J Clin Pharmacol 1994;38:427-32.

29. Korytkowski MT, Mokan M, Veneman TF, Mitrakou A, Cryer PE, Gerich JE. Reduced beta-adrenergic sensitivity in patients with type 1 diabetes and hypoglycemia unawareness. Diabetes Care 1998;21:1939-43.

30. Enoksson S, Caprio SK, Rife F, Shulman GI, Tamborlane WV, Sherwin RS. Defective activation of skeletal muscle and adipose tissue lipolysis in type 1 diabetes mellitus during hypoglycemia. J Clin Endocrinol Metab 2003;88:1503-11.

31. Cryer PE. Hypoglycemia-associated autonomic failure in diabetes. Am J Physiol Endocrinol Metab 2001;281:E1115-21.

32. Davis SN, Mann S, Galassetti P, et al. Effects of differing durations of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia in normal humans. Diabetes 2000;49:1897-903.

33. Davis SN, Tate D. Effects of morning hypoglycemia on neuroendocrine and metabolic responses to subsequent afternoon hypoglycemia in normal man. J Clin Endocrinol Metab 2001;86:2043-50.

34. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J. Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. Diabetes 1993;42:1233-7.

35. Ovalle F, Fanelli CG, Paramore DS, Hershey T, Craft S, Cryer PE. Brief twice-weekly episodes of hypoglycemia reduce detection of clinical hypoglycemia in type 1 diabetes mellitus. Diabetes 1998;47:1472-9.

36. Cryer PE. Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes 1994;43:1378-89.

37. Polinsky RJ, Kopin IJ, Ebert MH, Weise V. The adrenal medullary response to hypoglycemia in patients with orthostatic hypotension. J Clin Endocrinol Metab 1980;51:1401-6.

38. Hoeldtke RD, Boden G, Shuman CR, Owen OE. Reduced epinephrine secretion and hypoglycemia unawareness in diabetic autonomic neuropathy. Ann Intern Med 1982;96:459-62.

39. Bottini P, Boschetti E, Pampanelli S, et al. Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a nonselective defect. Diabetes 1997;46:814-23.

40. Hepburn DA, Patrick AW, Eadington DW, Ewing DJ, Frier BM. Unawareness of hypoglycaemia in insulin-treated diabetic patients: prevalence and relationship to autonomic neuropathy. Diabet Med 1990;7:711-7.

41. Stephenson JM, Kempler P, Perin PC, Fuller JH. Is autonomic neuropathy a risk factor for severe hypoglycaemia? The EURODIAB IDDM Complications Study. Diabetologia 1996;39:1372-6.

42. de Galan BE, Hoekstra JB. Glucose counterregulation in Type 2 diabetes mellitus. Diabet Med 2001;18:519-27.

43. Shamoon H, Friedman S, Canton C, Zacharowicz L, Hu M, Rossetti L. Increased epinephrine and skeletal muscle responses to hypoglycemia in non-insulin-dependent diabetes mellitus. J Clin Invest 1994;93:2562-71.

44. Levy CJ, Kinsley BT, Bajaj M, Simonson DC. Effect of glycemic control on glucose counterregulation during hypoglycemia in NIDDM. Diabetes Care 1998;21:1330-8.

45. Meneilly GS, Cheung E, Tuokko H. Counterregulatory hormone responses to hypoglycemia in the elderly patient with diabetes. Diabetes 1994;43:403-10.

46. Spyer G, Hattersley AT, MacDonald IA, Amiel S, MacLeod KM. Hypoglycaemic counter-regulation at normal blood glucose concentrations in patients with well controlled type-2 diabetes. Lancet 2000;356:1970-4.

47. Korzon Burakowska A, Hopkins D, et al. Effects of glycemic control on protective responses against hypoglycemia in type 2 diabetes. Diabetes Care 1998;21:283-90.

48. Ortiz Alonso FJ, Herman WH, Zobel DL, Perry TJ, Smith MJ, Halter JB. Effect of epinephrine on pancreatic beta-cell and alpha-cell function in patients with NIDDM. Diabetes 1991;40:1194-202.

49. Leese GP, Wang J, Broomhall J, et al. Frequency of severe hypoglycemia requiring emergency treatment in type 1 and type 2 diabetes: a population-based study of health service resource use. Diabetes Care 2003;26:1176-80.

50. Segel SA, Paramore DS, Cryer PE. Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. Diabetes 2002;51:724-33.

51. Henderson JN, Allen KV, Deary IJ, Frier BM. Hypoglycaemia in insulin-treated Type 2 diabetes: frequency, symptoms and impaired awareness. Diabet Med 2003;20:1016-21.

52. Akram K, Pedersen-Bjergaard U, Borch-Johnsen K, Thorsteinsson B. Recall of severe hypoglycaemic episodes and course of hypoglycaemia awareness in insulin-treated Type 2 diabetes in one year follow-up. Diabetologia 2003;46:A304.

53. Davis SN, Shavers C, Costa F, Mosqueda Garcia R. Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. J Clin Invest 1996;98:680-91.

54. McGregor VP, Banarer S, Cryer PE. Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia. Am J Physiol Endocrinol Metab 2002;282:E770-7.

55. Davis SN, Shavers C, Davis B, Costa F. Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. J Clin Invest 1997;100:429-38.

56. Raju B, McGregor VP, Cryer PE. Cortisol elevations comparable to those that occur during hypoglycemia do not cause hypoglycemia-associated autonomic failure. Diabetes 2003;52:2083-9.

57. Galassetti P, Mann S, Tate D, et al. Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. Am J Physiol Endocrinol Metab 2001;280:E908-17.

58. McGregor VP, Greiwe JS, Banarer S, Cryer PE. Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure. Diabetes 2002;51:1485-92.

59. McCall AL, Fixman LB, Fleming N, Tornheim K, Chick W, Ruderman NB. Chronic hypoglycemia increases brain glucose transport. Am J Physiol 1986;251:E442-7.

60. Uehara Y, Nipper V, McCall AL. Chronic insulin hypoglycemia induces GLUT-3 protein in rat brain neurons. Am J Physiol 1997;272:E716-9.

61. Kumagai AK, Kang YS, Boado RJ, Pardridge WM. Upregulation of blood-brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes 1995;44:1399-404.

62. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C. Adaptation in brain glucose uptake following recurrent hypoglycemia. Proc Natl Acad Sci U S A 1994;91:9352-6.

63. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ. Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus [see comments]. N Engl J Med 1995;333:1726-31.

64. Benzo CA. Minireview. The hypothalamus and blood glucose regulation. Life Sci 1983;32:2509-15.

65. Biggers DW, Myers SR, Neal D, et al. Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. Diabetes 1989;38:7-16.

66. Frizzell RT, Jones EM, Davis SN, et al. Counterregulation during hypoglycemia is directed by widespread brain regions. Diabetes 1993;42:1253-61.

67. McCrimmon RJ, Jacob RJ, Fan X, McNay EC, Sherwin RS. Effects of recurrent antecedent hypoglycaemia and chronic hyperglycaemia on brainstem extra-cellular glucose concentrations during acute hypoglycaemia in conscious diabetic BB rats. Diabetologia 2003;46:1658-61.

68. McNay EC, Sherwin RS. Effect of recurrent hypoglycemia on spatial cognition and cognitive metabolism in normal and diabetic rats. Diabetes 2004;53:418-25.

69. Segel SA, Fanelli CG, Dence CS, et al. Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. Diabetes 2001;50:1911-7.

70. Bingham EM, Dunn JT, Smith D, et al. Differential changes in brain glucose metabolism during hypoglycaemia accompany loss of hypoglycaemia awareness in men with type 1 diabetes mellitus. An ^{11C}-3-O-methyl-D-glucose PET study. Diabetologia 2005;48:2080-9.

71. Fagius J, Berne C. Changes of sympathetic nerve activity induced by 2-deoxy-D- glucose infusion in humans. Am J Physiol 1989;256:E714-20.

72. Amiel SA, Archibald HR, Chusney G, Williams AJ, Gale EA. Ketone infusion lowers hormonal responses to hypoglycaemia: evidence for acute cerebral utilization of a non-glucose fuel. Clin Sci Colch 1991;81:189-94.

73. Maran A, Cranston I, Lomas J, Macdonald I, Amiel SA. Protection by lactate of cerebral function during hypoglycaemia [see comments]. Lancet 1994;343:16-20.

74. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J. Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans. Diabetes 1994;43:1311-7.

75. Bischof MG, Mlynarik V, Brehm A, et al. Brain energy metabolism during hypoglycaemia in healthy and type 1 diabetic subjects. Diabetologia 2004;47:648-51.

76. De Galan BE, Van der Graaf M, Klomp D, Parkes LM, Heerschap A, Tack CJ. Feasibility study on the use of MRS to investigate human brain metabolism in vivo during hypoglycemia. Diabetes 2005;54:A155.

77. Cryer PE. Hypoglycaemia: The limiting factor in the glycaemic management of Type I and Type II Diabetes*. Diabetologia 2002;45:937-48.

78. Tattersall RB, Gill GV. Unexplained deaths of type 1 diabetic patients. Diabet Med 1991;8:49-58.

79. Campbell I. Dead in Bed Syndrome – A New Manifestation of Nocturnal Hypoglycemia. Diabet Med 1991;8:3-4.

80. Robinson RT, Harris ND, Ireland RH, MacDonald IA, Heller SR. Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. Diabetologia 2004;47:312-5.

81. Lindstrom T, Jorfeldt L, Tegler L, Arnqvist HJ. Hypoglycaemia and cardiac arrhythmias in patients with type 2 diabetes mellitus. Diabet Med 1992;9:536-41.

82. Agardh CD, Rosen I, Ryding E. Persistent vegetative state with high cerebral blood flow following profound hypoglycemia. Ann Neurol 1983;14:482-6.

83. Chalmers J, Risk MT, Kean DM, Grant R, Ashworth B, Campbell IW. Severe amnesia after hypoglycemia. Clinical, psychometric, and magnetic resonance imaging correlations. Diabetes Care 1991;14:922-5.

84. Perros P, Sellar RJ, Frier BM. Chronic pontine dysfunction following insulin-induced hypoglycemia in an IDDM patient. Diabetes Care 1994;17:725-7.

85. Casey E, Evans A, Krentz A, Watkins P, Hopkins D. Central pontine myelinolysis. An unusual complication of diabetes. Diabetes Care 1999;22:998-1000.

86. Musen G, Lyoo IK, Sparks CR, et al. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. Diabetes 2006;55:326-33.

87. Langan SJ, Deary IJ, Hepburn DA, Frier BM. Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. Diabetologia 1991;34:337-44.

88. Reichard P, Pihl M, Rosenqvist U, Sule J. Complications in IDDM are caused by elevated blood glucose level: the Stockholm Diabetes Intervention Study (SDIS) at 10-year follow up. Diabetologia 1996;39:1483-8.

89. Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial. Ann Intern Med 1996;124:379-88.

90. Austin EJ, Deary IJ. Effects of repeated hypoglycemia on cognitive function: a psychometrically validated reanalysis of the Diabetes Control and Complications Trial data. Diabetes Care 1999;22:1273-7.

91. Ferguson SC, Blane A, Perros P, et al. Cognitive ability and brain structure in type 1 diabetes: relation to microangiopathy and preceding severe hypoglycemia. Diabetes 2003;52:149-56.

92. Sima AA, Kamiya H, Li ZG. Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. Eur J Pharmacol 2004;490:187-97.

93. Pedersen-Bjergaard U, Pramming S, Thorsteinsson B. Recall of severe hypoglycaemia and self-estimated state of awareness in type 1 diabetes. Diabetes Metab Res Rev 2003;19:232-40.

94. Banarer S, Cryer PE. Sleep-related hypoglycemia-associated autonomic failure in type 1 diabetes – Reduced awakening from sleep during hypoglycemia. Diabetes 2003;52:1195-203.

95. Chen M, Daly M, Williams N, Williams S, Williams C, Williams G. Non-invasive detection of hypoglycaemia using a novel, fully biocompatible and patient friendly alarm system. BMJ 2000;321:1565-6.

96. Stocks AE. Can dogs help patients with hypoglycaemia? Diabetologia 2002;45:A79.

97. Fanelli CG, Epifano L, Rambotti AM, et al. Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. Diabetes 1993;42:1683-9.

98. Dagogo Jack S, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. Diabetes 1994;43:1426-34.

99. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA. Restoration of hypoglycaemia awareness in patients with long- duration insulin-dependent diabetes. Lancet 1994;344:283-7.

100. Fritsche A, Stefan N, Haring H, Gerich J, Stumvoll M. Avoidance of hypoglycemia restores hypoglycemia awareness by increasing beta-adrenergic sensitivity in type 1 diabetes. Ann Intern Med 2001;134:729-36.

101. Cryer PE. Hypoglycaemia: The limiting factor in the glycaemic management of Type I and Type II Diabetes*. Diabetologia 2002;45:937-48.

102. Jorgensen HV, Pedersen-Bjergaard U, Rasmussen AK, Borch-Johnsen K. The impact of severe hypoglycemia and impaired awareness of hypoglycemia on relatives of patients with type 1 diabetes. Diabetes Care 2003;26:1106-9.

103. Anderson JH Jr, Brunelle RL, Koivisto VA, Trautmann ME, Vignati L, DiMarchi R. Improved mealtime treatment of diabetes mellitus using an insulin analogue. Multicenter Insulin Lispro Study Group. Clin Ther 1997;19:62-72.

104. Brunelle BL, Llewelyn J, Anderson JH, Jr., Gale EA, Koivisto VA. Meta-analysis of the effect of insulin lispro on severe hypoglycemia in patients with type 1 diabetes. Diabetes Care 1998;21:1726-31.

105. Home PD, Lindholm A, Riis A. Insulin aspart vs. human insulin in the management of long-term blood glucose control in Type 1 diabetes mellitus: a randomized controlled trial. Diabet Med 2000;17:762-70.

106. Lalli C, Ciofetta M, Del Sindaco P, et al. Long-term intensive treatment of type 1 diabetes with the short-acting insulin analog lispro in variable combination with NPH insulin at mealtime. Diabetes Care 1999;22:468-77.

107. Wang F, Carabino JM, Vergara CM. Insulin glargine: a systematic review of a long-acting insulin analogue. Clin Ther 2003;25:1541-77, discussion.

108. Ratner RE, Hirsch IB, Neifing JL, Garg SK, Mecca TE, Wilson CA. Less hypoglycemia with insulin glargine in intensive insulin therapy for type 1 diabetes. U.S. Study Group of Insulin Glargine in Type 1 Diabetes. Diabetes Care 2000;23:639-43.

109. Yki-Jarvinen H, Dressler A, Ziemen M. Less nocturnal hypoglycemia and better post-dinner glucose control with bedtime insulin glargine compared with bedtime NPH insulin during insulin combination therapy in type 2 diabetes. HOE 901/3002 Study Group. Diabetes Care 2000;23:1130-6.

110. Hermansen K, Madsbad S, Perrild H, Kristensen A, Axelsen M. Comparison of the soluble basal insulin analog insulin detemir with NPH insulin: a randomized open crossover trial in type 1 diabetic subjects on basal-bolus therapy. Diabetes Care 2001;24:296-301.

111. Boland EA, Grey M, Oesterle A, Fredrickson L, Tamborlane WV. Continuous subcutaneous insulin infusion. A new way to lower risk of severe hypoglycemia, improve metabolic control, and enhance coping in adolescents with type 1 diabetes. Diabetes Care 1999;22:1779-84.

112. Bode BW, Steed RD, Davidson PC. Reduction in severe hypoglycemia with long-term continuous subcutaneous insulin infusion in type I diabetes. Diabetes Care 1996;19:324-7.

113. Linkeschova R, Raoul M, Bott U, Berger M, Spraul M. Less severe hypoglycaemia, better metabolic control, and improved quality of life in Type 1 diabetes mellitus with continuous subcutaneous insulin infusion (CSII) therapy; an observational study of 100 consecutive patients followed for a mean of 2 years. Diabet Med 2002;19:746-51.

114. Kanc K, Janssen MM, Keulen ET, et al. Substitution of night-time continuous subcutaneous insulin infusion therapy for bedtime NPH insulin in a multiple injection regimen improves counterregulatory hormonal responses and warning symptoms of hypoglycaemia in IDDM. Diabetologia 1998;41:322-9.

115. Wiethop BV, Cryer PE. Alanine and terbutaline in treatment of hypoglycemia in IDDM. Diabetes Care 1993;16:1131-6.

116. Saleh TY, Cryer PE. Alanine and terbutaline in the prevention of nocturnal hypoglycemia in IDDM. Diabetes Care 1997;20:1231-6.

117. Porcellati F, Pampanelli S, Rossetti P, et al. Counterregulatory hormone and symptom responses to insulin-induced hypoglycemia in the postprandial state in humans. Diabetes 2003;52:2774-83.

118. De Galan BE, de Mol P, Wennekes L, Schouwenberg BJ, Smits P. Preserved sensitivity to beta2-adrenergic receptor agonists in patients with type 1 diabetes mellitus and hypoglycemia unawareness. J Clin Endocrinol Metab 2006;91:2878-81.

119. Hvidberg A, Rasmussen MH, Christensen NJ, Hilsted J. Theophylline enhances glucose recovery after hypoglycemia in healthy man and in type I diabetic patients. Metabolism 1994;43:776-81.

120. Watson JM, Sherwin RS, Deary IJ, Scott L, Kerr D. Dissociation of augmented physiological, hormonal and cognitive responses to hypoglycaemia with sustained caffeine use. Clin Sci (Lond) 2003;104:447-54.

121. De Galan BE, Tack CJ, Lenders JW, et al. Theophylline improves hypoglycemia unawareness in type 1 diabetes. Diabetes 2002;51:790-6.

122. De Galan BE, Tack CJ, Lenders JW, Lutterman JA, Smits P. Effect of 2 weeks of theophylline on glucose counterregulation in patients with type 1 diabetes and unawareness of hypoglycemia. Clin Pharmacol Ther 2003;74:77-84.

123. Watson JM, Jenkins EJ, Hamilton P, Lunt MJ, Kerr D. Influence of caffeine on the frequency and perception of hypoglycemia in free-living patients with type 1 diabetes. Diabetes Care 2000;23:455-9.

124. Bingham E, Hopkins D, Pernet A, Reid H, MacDonald IA, Amiel SA. The effects of KATP channel modulators on counterregulatory responses and cognitive function during acute controlled hypoglycaemia in healthy men: a pilot study. Diabet Med 2003;20:231-7.

125. Gabriely I, Hawkins M, Vilcu C, Rossetti L, Shamoon H. Fructose amplifies counterregulatory responses to hypoglycemia in humans. Diabetes 2002;51:893-900.

126. Gabriely I, Shamoon H. Fructose normalizes specific counterregulatory responses to hypoglycemia in patients with type 1 diabetes. Diabetes 2005;54:609-16.

127. Cox DJ, Clarke WL, Gonder-Frederick L, et al. Accuracy of perceiving blood glucose in IDDM. Diabetes Care 1985;8:529-36.

128. Cox DJ, Gonder Frederick LA, Lee JH, Julian DM, Carter WR, Clarke WL. Effects and correlates of blood glucose awareness training among patients with IDDM. Diabetes Care 1989;12:313-8.

129. Cox DJ, Gonder Frederick L, Julian DM, Clarke W. Long-term follow-up evaluation of blood glucose awareness training. Diabetes Care 1994;17:1-5.

130. Kinsley BT, Weinger K, Bajaj M, et al. Blood glucose awareness training and epinephrine responses to hypoglycemia during intensive treatment in type 1 diabetes. Diabetes Care 1999;22:1022-8.

131. Ter Braak EW, de Valk HW, de la Bije YF, van der Laak MF, van Haeften TW, Erkelens DW. Response to training in blood glucose awareness is related to absence of previous hypoglycemic coma. Diabetes Care 2000;23:1199-200.

132. Broers S, le Cessie S, van Vliet KP, Spinhoven P, van der Ven NC, Radder JK. Blood Glucose Awareness Training in Dutch Type 1 diabetes patients. Short-term evaluation of individual and group training. Diabet Med 2002;19:157-61.

Netherlands
The Journal of Medicine

REVIEW

Obstructive sleep apnoea syndrome and genes

A. Kaparianos, F. Sampsonas, K. Karkoulias, K. Spiropoulos*

Department of Internal Medicine, Division of Pneumology, University Hospital of Patras
26500 Rio-Patras, Greece, *corresponding author: tel.: +30-261099 95 23, fax: +30-2610999523,
e-mail: k-spiropoulos@hotmail.com

ABSTRACT

Obstructive sleep apnoea (OSA) is a complex disease entity strongly influenced by genetic factors, especially those that affect obesity and fat distribution, upper airway muscle tone, craniofacial morphology, ventilatory control and sleep, giving rise to the OSA phenotype. OSA can also be considered a metabolic syndrome which adversely affects multiple organ systems, especially the cardiovascular system and the brain. The most widely used clinical marker for the diagnosis of OSA is the apnoea-hypopnoea index, calculated by polysomnography. A percentage of 35 to 40% of its variance can be attributed to genetic factors. Therefore, the identification and elucidation of the genes implicated in the pathogenesis of OSA becomes a matter of extensive research and could lead to the development of therapeutic agents that can have a beneficial effect on the natural course of OSA.

KEYWORDS

Genetic susceptibility, OSA, polymorphism

INTRODUCTION

Obstructive sleep apnoea (OSA) is defined by a constellation of signs and symptoms; specifically, the occurrence of repetitive episodes of complete or partial obstruction of the upper airway during sleep, usually in association with loud snoring and daytime sleepiness. Such episodes are often associated with arousals, sleep fragmentation, intermittent hypoxaemia and hypercapnia, and nocturnal hypertension.[1,2] Associated nocturnal symptoms include restlessness, excessive salivation and sweating, nocturia, and gastro-oesophageal reflux. The patient frequently wakes in the morning with a headache and dry mouth or throat. OSA is now recognised to occur commonly, affecting 2 to 3% of children,[3] 2 to 4% of middle-aged adults[4] and 10% of the elderly population. Among people over 55 years of age, 30 to 60% meet the polysomnographic diagnostic criterion of an apnoea-hypopnoea index (AHI) of ≥5.[2] Its association with several chronic health conditions, particularly obesity, hypertension, diabetes, and cardiovascular diseases,[5-7] has underscored the broad public health importance of this condition. An elucidation of the aetiology of OSA, and the extent to which the disorder is due to genetic factors, is needed to better develop screening and treatment approaches. Very important is the role of apnoea-hypopnoea index (AHI), which is simply a count of the number of apnoeas and hypopnoeas per hour of sleep.[8] Apnoea is defined as a pause of at least ten seconds in the oral-nasal flow of air, despite the movement of the chest or abdomen, which leads to a reduction in O_2 saturation (≥2.5%) and awakening, while hypopnoea occurs when mild obstruction leads to a decrease in air flow by 50%. This index may be moderately correlated with various indices of night-time oxygen desaturation and sleep fragmentation measured using overnight polysomnography, which includes recording of oronasal flow (thermocouples), thoraco-abdominal movements (strain gauges), electrocardiogram, submental electromyogram, electro-oculogram, electroencephalogram (C4-A1, C3-A2) and transcutaneous SaO_2. Subjects are often considered 'diseased' if the AHI exceeds the threshold value of 5.[9]

As with blood pressure, increasing values of AHI indicate increasing disease severity.[10] Most studies of the genetics of OSA have used the AHI as the major disease-defining variable, and these studies have demonstrated significant familial aggregation. Advantages of the use of AHI are simplicity and high night-to-night reproducibility,[11] whereas its major disadvantages include the between-laboratory variability in measurement technique, the lack of information it provides on the severity of individual events (duration, associated hypoxaemia and arousal) and its uniformity regarding the functional and physiological impact of the disorder.[10] OSA seems to be a complex disorder that includes multiple genes, environmental influences, and developmental factors. Specific gene

products may more directly influence lower level phenotypes than higher level phenotypes. Traits such as facial and head form, ventilatory chemosensitivity, load compensation, sympathetic nervous system activity, connective tissue laxity, muscle fatigability and central obesity are possible intermediate phenotypes for OSA. Examples of low level phenotypes include hormone levels or receptor subtypes.[10]

Inheritance and observed aggregation within families

Descriptive reports of families with multiple affected members[12,13] show that there is likely to be role for inheritance apart from familial influences related to obesity. Preliminary results from segregation analysis have further defined the likely magnitude of genetic influences. Relatives of patients with the sleep apnoea-hypopnoea syndrome reported snoring, daytime sleepiness and had more apnoeas and hypopnoeas, arousals from sleep, poorer sleep quality and more oxygen desaturations compared with relatives of controls.[14] Another study[15] calculated the estimated risk ratios for relatives of patients with OSA and found that they were increased. The risk ratio for first-degree relatives was 2.0 for parents and 1.9 for siblings. For second-degree relatives (half-sibs, uncles/aunts, grandparents) the estimated risk ratios were 1.9, 1.3 and 1.3, respectively, with the first two being significant. The estimated risk ratio for cousins was 1.3, which was also statistically significant. The more severely affected group (continuous positive airway pressure (CPAP)-treated) shows, in general, somewhat higher risk ratios. OSA was reported in members of the same and different generations. It was found in children as well as adults, and in obese and nonobese family members. One report cited the co-occurrence of OSA, seizures and anosmia in affected family members as suggesting an inherited syndrome.[16]

Twin studies and familial clustering of snoring

First of all, the concordance for snoring was greater between monozygotic (MZ) twins than between dizygotic (DZ) twins, suggesting a role for inheritance.[17] Recent twin studies have also shown higher concordance in monozygotic than in dizygotic twins for habitual snoring.[18] Furthermore, habitual snoring, excessive daytime sleepiness, and snorting, gasping, or apnoeas were reported two or four times more frequently among the first-degree relatives of patients with OSA than among control subjects.[19] In another study a significant relationship was demonstrated between family history of snoring and self-reported snoring.[20] Risk of snoring was increased approximately threefold when at least one first-degree relative was reported to be a snorer, and increased fourfold when both parents were reportedly snorers. First-degree relatives of patients with OSA have consistently been shown to be at increased risk. Familial aggregation is generally explained by the fact that most risk factors involved in the pathophysiology of OSA are, to a large extent, genetically determined.[16,21-24] The major risk factors for OSA include obesity, ventilatory control abnormalities, and craniofacial dysmorphism (disproportionate craniofacial anatomy).

AHI and reported frequency within families

There were studies in which the definition of the disease was based on various threshold values of the AHI. In the studies the prevalence of OSA among first-degree relatives of probands with OSA varied from 21% (in the Cleveland Family Study) to as high as 84%.[21] When OSA was defined by an AHI >5 occurring with daytime tiredness or sleepiness and one other symptom, prevalence among first-degree relatives was 84%.[21] Changing the definition by requiring an AHI >15 and the occurrence of daytime sleepiness reduced the prevalence to 13%. This remark emphasises the importance of carefully considering how phenotype is defined. The Cleveland study also found an association between sudden unexpected death in infancy and OSA, suggesting that there are some families that may be predisposed to both syndromes.[25]

Results of racial, sex and ethnic variation in AHI and BMI

Obesity is the most characteristic feature of OSA in both European-American and African-American adults, and is most commonly measured by an elevated body mass index (BMI). Although relatively little is known about OSA in non-European populations, emerging data from the United States suggest that both old and young African-Americans have higher levels of AHI than European-Americans. Obesity is more prevalent and is more epidemic in African-Americans than in European-Americans, with the greatest ethnic differences observed for females. OSA appears to present at a younger age in African-Americans than European-Americans, and may also be more severe. Hypertensive end organ disease (kidney and cerebral vascular disease) and cardiovascular mortality are two- to threefold more prevalent among African-Americans than European-Americans. Higher levels of AHI are seen in African-Americans than Caucasians, particularly among case families.[26] Elderly African-Americans were at an approximately twofold increased risk for sleep apnoea compared with elderly Caucasians, and also had more severe sleep apnoea. In the Cleveland family study, racial differences were most prominent in individuals <25 years of age and even higher among children <13 years (odds ratio >3.0).[27] Racial variations may be due to variations in upper airway anatomy and other physiological factors. Anatomic risk factors in African-Americans appeared to be related to increased upper airway soft tissue rather than bony features that reduce airway size. It has also been shown that the familial aggregation of OSA in Caucasian males may be largely determined by influences related to obesity. Another observed difference was that Pacific Islanders and Maori living in New Zealand suffer from more severe OSA compared with individuals of European descent.[28] These racial effects were attributed

to group differences in neck size and body mass. Further light needs to be shed on the extent to which environmental or cultural *vs* genetic factors explain such difference in underlying pathogenetic mechanisms for OSA.

Strong genetic effects underlying the BMI have been shown by studies conducted on Nigerian, Jamaican, and African-American families, with the heritability of adult BMI ranging from 48 to 58%.[29] The heritability for AHI has also been shown to have a strong genetic predisposition, in a study conducted in African-American families it was around 32%.[26] A wide genomic scan showed some evidence of linkage for both AHI and BMI to a broad region on chromosome 8q, namely 8q24. It could represent a locus affecting AHI and BMI independently. Other regions showing linkage with obesity-related phenotypes include chromosome 4q23, connected with abdominal subcutaneous fat and trunk-to-extremity skinfold ratio. The 4q23 region contains the intestinal fatty acid-binding protein 2 (*FABP2*) and uncoupling protein 1 (*UCP1*) genes, which have been associated with BMI, percentage of body fat, abdominal fat, and weight loss.[26] The 8q21 region linked BMI and leptin levels, and the 10q26 region associated BMI levels with measures of abdominal fat. The chromosome 8q22 region also contains a core-binding factor (runt domain, P subunit) gene (*CBFA2T1*), which has been previously associated with BMI, percentage of body fat, and waist-and-hip circumference in a study of Pima Indians.[26] The 10q24–26 region contains the ponsin (*SH3D5*) and P-2A-adrenergic receptor (*ADRA2A*) genes, which have been associated with measures of obesity or fat distribution. QRS was also linked with loci on chromosomes 2p, 7p, and 12p. Regions that showed strong linkage with AHI were located on chromosomes 1p, 2p, 12p, and 19p.[26] It therefore seems that although they appear to be independent parameters, two important markers associated with OSA (AHI and BMI) have both shared and unshared genetic determinants and the analysis of the various genetic loci linked with them, through mapping efforts, could give more information on the genetics of OSA and its phenotypes

Obesity, fat distribution and implicated genes

Obesity appears to increase risk of OSA approximately 10 to 14-fold.[30,31] In contrast, weight loss may reduce the severity of the condition.[32] Obesity may lead to OSA through fat deposition in upper airway tissues, reducing nasopharyngeal caliber and/or from hypoventilation occurring in association with reduced chest wall compliance. Twin studies also showed that 70% of the variance in obesity within the general population can be attributed to genetic factors.[23,24] Familial studies of abdominal visceral fat reveal that the familial transmission reaches >50% of the age, sex and total body fat adjusted variance.[24] Obesity is believed to be secondary to abnormalities in autonomic, endocrine, and hypothalamic function which, in turn, are associated with genetic factors that influence metabolic rate, fat storage, and eating behaviour. About a quarter of the between-twin variability in regional body fat distribution may be influenced by genetic factors.[24] Hence, upper-body obesity may be a relatively greater risk factor for OSA than total body fat mass. The heritability of the amount of upper body fat or the level of upper body fat relative to lower body fat ranges from approximately 30 to 50% of the phenotype's age, sex and total body fat adjusted variance.[24] Even relatively nonobese individuals with OSA may have regional excess fat deposition, especially in the anterolateral upper airway.[33]

The coaggregation of OSA, central obesity, hypertension, and type 2 diabetes suggests that OSA may be part of a 'metabolic' syndrome, which may be largely influenced by genes that have an effect on insulin resistance and body fat distribution. Candidate genes for obesity are therefore relevant for studies of the genetics of OSA both because of the prominence of obesity in the OSA phenotype, and because of the potential impact of these genes on the expression of other traits of potential relevance to OSA. Previous genetic studies of obesity have not evaluated OSA, which may occur in as many as 66% of obese individuals. The term 'syndrome Z' has been introduced for the combination of hypertension, central obesity, insulin resistance, hyperlipidaemia, and OSA.[34] Some studies have shown combined metabolic abnormalities in patients with obesity (metabolic syndrome or syndrome X), and sleep deprivation itself has been shown to have metabolic consequences.[35] It is plausible that OSA may increase the risk of obesity. For example, OSA causes sleep fragmentation and sleepiness, effects that may promote weight gain through reduced physical activity and hypercytokinaemia.[36]

There are a number of studies showing the pleiotropic effects of leptin (an adipose-derived circulating hormone), not only in appetite regulation but also in lung growth and respiratory control as well as in sleep architecture. A genome wide scan conducted on ten extended Mexican-American families showed an established linkage between serum leptin levels and areas on chromosomes 2 and 8.[37] These regions respectively encompass genes encoding for pro-opiomelanocortin (POMC), which may be important in appetite regulation, and for X-3-adrenergic receptor (ADRB3), which may influence the regulation of energy expenditure. A more recent family study in Germany has additionally implicated mutations in the melanocortin-4 receptor gene (MC4-R) in extreme and moderate obesity.[38] A number of candidate genes for obesity (e.g. leptin, adenosine deaminase and melanocortin-4 receptor) are expressed in a variety of tissues and brain sites important in the regulation of breathing.[39,40] In knockout mice models leptin deficiency causes depressed ventilatory responses to hypercapnia in both wakefulness and sleep.[41] Finally, leptin administration also influences sleep architecture in rats.[42]

Craniofacial morphology in the aetiology of OSA

Craniofacial morphology is very important since it determines the anatomy and the diameter of the upper

airways. OSA occurs when the size of the upper airway is reduced.[43] Structural abnormalities that cause this are reduction of the anteroposterior dimension of the cranial base,[44] reduction of the size of the posterior and superior airway spaces,[45] inferior displacement of the hyoid,[46] elongation of the soft palate, macroglossia, adenoidotonsillar hypertrophy and increased vertical facial dimension, with a disproportionate increase in the lower facial height.[44] Retrognathia and micrognathia have also been linked with OSA, although the link was not as strong.[47] Also a brachycephalic head form often creates a problem since it is associated with sudden unexpected death in infancy and an increased risk of OSA in Caucasians while in people of African descent, this head form is uncommon, and therefore does not appear to increase risk of OSA. Finally, OSA is common in individuals with Down's syndrome, which is commonly associated with a number of craniofacial dysmorphisms, a clue that provides another link between genes affecting craniofacial morphology and OSA. Another study in 60 MZ and 40 DZ twins estimated the heritability of a number of measures of craniofacial structure. The heritability of one of these, the cephalic index, was extremely high (0.90 in males, 0.70 in females). Heredity appeared to account for 40% of the variability of dental and facial characteristics associated with malocclusions. In humans, micrognathia can be found in a myriad of chromosomal deletion syndromes, suggesting that genes affect and alter normal craniofacial growth.

Studies in mice have shown that deficiency in transforming growth factor-X2,[46] endothelin-1,[47] retinoic acid receptor-aZ[48] and collagen gene mutations (types II and XI) leads to various craniofacial abnormalities, including retrognathia and micrognathia. Various other genetic syndromes are known to cause problems in the organisation of the extracellular matrix and are associated with craniofacial dysmorphism and upper airway connective tissue laxity. One of these syndromes, the Marfan syndrome, causes abnormalities in fibrillin and may contribute to both.[49] A recent[50] study showed the volume of the lateral pharyngeal walls, tongue and total soft tissue demonstrated significant levels of heritability and that heritability of the upper airway soft tissue structures is found in normal subjects and patients with apnoea.

Ventilatory control and chemoreceptor sensitivity

Inherited abnormalities of ventilatory control may predispose to obstructive or central sleep apnoea or both by influencing ventilation during sleep and increasing the propensity to upper airway collapse. Altered ventilatory drive may participate in sleep apnoea and periodic breathing, while ventilatory control instability could cause blunted or augmented chemosensitivity.[51,52] This notion is supported by the demonstration that the degree of oxygen desaturation is the greatest and the duration of apnoeas the longest in subjects with OSA in whom ventilation in response to hypoxia during wakefulness is the most blunted. A study by El Bayadi *et al*. demonstrated blunted ventilatory responses to progressive eucapneic hypoxia ventilatory challenges in all five of the affected subjects studied.[16] Thus, in this family, the underpinnings of OSA may have been associated with inherited abnormalities in the control of ventilation. A genetic basis for the chemoresponse to blood oxygen saturation is suggested by several twin studies that have demonstrated similarities in ventilatory responses to hypoxia or hyperoxia to be greater in monozygotic than in dizygotic twins.[53,54] The variance of responses using a single-breath hypoxic stimulus was greater within dizygotic pairs than in monozygotic twins.[54] Heritability estimates for chemoresponsivity to oxygen saturation levels vary between approximately 30 and 75%, suggesting a substantial contribution of inheritance to this trait.[54] Evidence for a role for genetics in the ventilatory response to hypercapnia in humans is less consistent. Members of OSA families significantly demonstrated a reduced ventilatory response to progressive eucapnic hypoxia measured during wakefulness compared with members of control families.[55] The finding was a significantly greater increase in ventilatory impedance with inspiratory resistive loading in OSA family members compared with control subjects. The familial aggregation of OSA may in some instances be based on inherited abnormalities in ventilatory control, perhaps related to chemoregulation and/or load compensation. The upper airway of genetically susceptible individuals appears vulnerable to excess collapsibility during conditions of mild inspiratory loading. This may occur especially during sleep as the balance between upper airway and chest wall activation changes or intrathoracic airway pressure during inspiration becomes more negative. Also, it has been shown that tidal volume is reduced in relatives of apnoea sufferers under resistive loading.[56]

There are numerous case reports of children with frequent apnoeas and daytime hypoventilation that appear attributable to severe chemoregulatory dysfunction, manifest as profound blunting of the hypercapnic and hypoxic ventilatory responses.[57] Developmental abnormalities of the brainstem or cerebral cortex have been found in some of these cases. It is worth noting that Hirschsprungs disease, a congenital disorder characterised by intestinal dysmotility and absence of myenteric and submucosal ganglia in the distal bowel, may occur in as many as 50% of cases of idiopathic congenital central hypoventilation (CCH), known as Ondine's curse.[58] Mutations of both the RET proto-oncogene, encoding a receptor tyrosine kinase thought to be involved in neural crest migration and proliferation, and the RET ligand, glial cell line-derived neurotrophic factor (GDNF),[59] have been described in children with Hirschsprungs disease, and in CCH occurring in association with Hirschsprungs disease.[60,61] More recent studies, however, implicate the PHOX2b gene as the most important cause of this syndrome. PHOX2b is mapped to chromosome

4p12 and encodes a highly conserved homeobox domain transcription factor (314 amino acids), with two short and stable polyalanine repeats of nine and 20 residues.[62] It has an early embryological function as a transcriptional activator in promotion of pan-neuronal differentiation including upregulation of proneural gene and mammalian achaete-scute homologue-1 (MASH1) expression, and expression of motoneural differentiation.[63] Likewise, genes pertinent to early embryological development of the autonomic nervous system (ANS) and their effect on respiratory drive are now a major area of interest and active research, such as mammalian achaete-scute homologue-1 (MASH1), bone morphogenic protein-2 (BMP2), engrailed-1 (EN1) TLX3, endothelin-converting enzyme-1 (ECE1), endothelin-1 (EDN1), PHOX2a and PHOX2b.[63] Two studies[62,63] showed that children with CCH are heterozygous for the PHOX2b polyalanine expansion mutation, although the frequency observed in the incidence of this mutation as well as in the incidence of any PHOX2b mutation differed (97 and 98.5% in the Weese-Mayer *et al*. study, 62 and 69% in the Amiel *et al*. study). Association between the polyalanine repeat mutation length and severity was also found,[63] while the Amiel *et al*. study found no such association.[62] The Amiel *et al*. study suggested that the mutation arises *de novo* while the later study suggests that it is heritable, in an autosomal dominant fashion. These associations suggest that CCH syndromes may sometimes be caused by abnormalities in migration of neural crest cells to central respiratory control centres and can provide critical information concerning the effect of genes on respiratory drive and its dysfunction. Other genes involved in the endothelin signalling pathway (endothelin B receptor gene, EDNRB and endothelin 3 gene, EDN3) have also been implicated in Hirschsprungs disease and could be considered candidate genes for CCH syndromes and sleep apnoea.[64] Other loci of interest may be identified on chromosome 15, mutations of which may result in a number of somatic abnormalities (e.g. Prader–Willi syndrome) as well as OSA.

Heterozygous and homozygous RET knockout mice, who survived only briefly, demonstrated reductions in hypercapnic ventilatory responses.[65] Endothelin-1 (ET-1), a potent vasoactive peptide, may also participate in control of ventilation. In a knockout mouse model, absence of ET-1 results in respiratory failure, ventilatory control abnormalities, craniofacial abnormalities and hypertension, characteristics remarkably similar to traits found in OSA. Mutant mice deficient in ET-1 have impaired ventilatory responses to both hypoxia and hypercapnia.[66] A zinc finger protein, namely Krox-20, has been identified which affects the development of the hindbrain. If deleted by homologous recombination in mice, they demonstrate slow respiratory frequencies and long apnoeas.[67] Loss of another factor, brain-derived neurotrophic factor (BDNF), results in reduced survival of neurons in the nodosepetrosal ganglion.[68] Homozygous mice demonstrated irregular and depressed ventilation, including spontaneous apnoeas, and abnormalities in chemoregulation specifically related to hyperoxia but not to hypercapnia. Nonlethal alterations in the genetic control of neural growth factors may contribute to phenotypic variations in ventilatory traits.[67] A small cluster of genes seem to play the major role in inheritance. These are candidate genes that encode neuroreceptors (e.g. glycine receptor, glutamate receptor) and genes that influence the postnatal development of the lung (e.g. basic fibroblast growth factor, bFGF), in the mouse model described by Tankersley.[68] Another link made between respiratory control and genetic loci was one pointing to the 8q22 chromosomal region. It contains three genes for carbonic anhydrase (CA) isoenzymes: CA1, CA2, and CA3. The roles of CA in modulating respiratory control, and the role of CA inhibitors as potential treatment for conditions with underlying respiratory instability, including sleep periodic breathing and sleep apnoea, have been the subjects of numerous animal and human studies.[69-71]

Sleep regulation, REM sleep, orexins and OSA

Some of the most exciting work on sleep-wake control has come from recent studies of narcolepsy (cataplexy, REM-onset sleep and hypersomnolence). Studies resulted in the discovery that canine narcolepsy, which is transmitted as a single autosomal recessive trait with full penetrance, is caused by mutations in one of the receptors for the newly discovered lateral hypothalamic neuropeptides, the hypocretin-1 (HCRT-1) and hypocretin-2 (HCRT-2) (also called orexins A and B, respectively),[72] two polypeptides that are ligands for two G protein-coupled receptors in the brain. At around the same time, mice with targeted disruption of the hypocretin precursor (preprohypocretin) gene were shown to have periods of behavioural arrest and EEG patterns that resemble human narcolepsy.[73] These findings in animals have now been extended to human beings and most of the narcolepsy-cataplexy patients studied have been shown to have low or undetectable hypocretin in their CSF.[74,75] Few post-mortem studies of the human brain have been conducted, but these studies have shown that patients with narcolepsy have much lower than normal hypocretin levels in the brain.[76,77]

So far, only one case of narcolepsy has been associated with a mutation in the gene that encodes preprohypocretin.[76] This case is unusual in that the onset of narcolepsy was at a very early age (cataplexy at age 6 months). The mutation in the preprohypocretin gene (a polar substitution in the hydrophobic core of its molecule, specifically arginine insertion in the polyleucine stretch of neutral, hydrophobic amino acids) results in abnormal trafficking of the mutant peptide precursor.[76] Mutations in the hypocretin 2 receptor have been identified in canine narcolepsy and disruption of the prepro-orexin /hypocretin ligand gene results in both an animal model of narcolepsy and sporadic cases of the human disease. Orexin neurons have been demonstrated to have widespread projections to areas

in the ascending cortical activating system, including the tuberomamillary nucleus, locus ceruleus, the dorsal and median raphe and pedunculopontine nuclei.[73] The pedunculopontine nuclei are thought to be especially critical to the control of REM sleep. Abnormalities in orexin genes, or genes coding for their receptors, could be relevant to studies of OSA because of the potential impact of these neuropeptides on arousal and muscle tone, both of which influence the behaviour of respiratory systems, and/ or because of the close proximity of these neurons to central respiratory control centres, with potential interactions between arousal and respiratory centres.

An alternative way to access the molecular biology of respiration is to characterise the genetic variation involved in individual differences in the control of respiratory behaviour. A large twin-sibling study[77] tested the heritability of 24-hour respiration rate and its genetic linkage through a whole genome scan. Four genomic regions were identified as having a high likelihood of harbouring loci that influence respiration rate, in particular loci 10q26, 22q12, 3q27 and 7p22. Positional candidate genes with the strongest evidence of linkage that are implicated in this study are the glial cell line-derived neurotrophic factor family receptor alpha-1 gene (GRFA-1, implicated in CCH), fibroblast growth factor receptor 2 gene (with its major role in craniosynostosis syndromes) and the homeobox genes HMX2 and EMX2 (with an as yet unknown function in respiratory rhythmogenesis) in the proximity of 10q, the adenosine A_{2A} and A_2 receptor genes (ADORA2A and ADORA2L respectively, known to affect REM sleep and respiratory drive) in the proximity of 22q12 and the 5-HT receptor 3C gene (HTR3C, the role of serotonin is discussed further below). It should also be noted that another finding of this study is that the heritability of respiration rate was found to be moderate during the daytime (41 to 50%), but to sharply increase at night (81%).[77] This shift in genetic architecture suggests that respiration rate is under more control during sleep than during awake periods. This makes sense since many environmental factors such as speech or physical activity impact respiration during the daytime, whereas, during sleep, respiratory frequency will be a more pure reflection of intrinsic rhythmogenesis by the brain stem.

Polymorphisms in serotonin receptor, transporter genes and OSA

Patency of the human upper airway is mostly maintained by muscle activation and soft tissue structures. The activity of the muscles responsible for maintaining patency of the upper airway is increased during inspiration, thus stiffening and dilating the upper airway and acting to counteract the collapsing influence of negative airway pressure.[78] During sleep there is a loss of both tonic premotor input (and neuromuscular compensation) and reflex-driven muscle activation leading to a large decrement in electromyogram and ultimately airway collapse. 5-HT plays an important role in the patency of the upper airway. 5-HT excites upper airway dilator motor neurons in adults[79,80] and provides intrinsic excitation of brainstem motor neurons in un-anaesthetised animals.[81] The activity of neurons supplying 5-HT to motor neurons declines during sleep.[82,83] Furthermore, pretreatment of upper airway dilator motor neurons with 5-HT reduces sleep state-dependent suppression in upper airway dilator muscle activity.[84]

5-HT acts through a large family of receptors.[85] The 5-HT 2A/2C receptor subtype plays an important role in the maintenance of upper airway stability and normal breathing in obesity. 5-HT 2A is the predominant excitatory 5-HT receptor subtype at the hypoglossal motor neurons.[86] The excitatory effects of the 5-HT 2C receptor are of a lower magnitude.[86] Based on these data, polymorphisms in the 5-HT 2A/2C receptor genes were studied in order to investigate whether or not they are associated with OSA, but the results showed no significant relationship.[87] Synaptic 5-HT is inactivated by presynaptic reuptake, which is mediated by the serotonin transporter. The aim of another study was the polymorphism of the serotonin transporter gene, the associated alterations in serotonin level and their importance in OSA.[88] The serotonin transporters are coded by the serotonin transporter gene (STG) that is located on chromosome 17q12. A polymorphism of the gene coding for the serotonin transporter has been identified,[89,90] and two polymorphisms, VNTR (variable-number-tandem-repeats of 17 bp sequence in the second intron and has several alleles) and 5-HTTLPR, have been described. The function of VTNR is thought to affect enhancer function and thus transcription of the gene. 5-HTTLPR (5-HTT gene-linked polymorphic region) is a deletion insertion polymorphism located at the 5'-flanking regulatory region of the STG and creates short (S) and long (L) alleles. The uptake of serotonin in cells homozygous for the L form (or L/L) of the promoter polymorphism was found to be 1.9 to 2.2 times greater than that in cells carrying one or two endogenous copies of the S (or S/L, or S/S) allele. That is, S allele corresponds to low serotonin uptake activity.[91]

Although the study did not reveal any significant difference between the patients and controls regarding the genotypes and allele frequencies, there were significant differences between the results of male and female patients as well as between male patients and male controls.[88] These findings may suggest a genetic predisposition to OSA, especially in male patients, which results in an alteration in the activity of serotonergic system. These results are supported with the finding that there is a five- or sixfold increased risk of obstructive sleep apnoea in men compared with women according to sleep laboratory data, and two- or threefold increased risk in men *vs* women according to community-based studies.[92,93]

The presence of the S allele is associated with decreased 5-HT reuptake, which, in turn, results in longer serotonergic

activity. The frequency of S allele and S/S genotype was less frequent in male patients than in male controls and female patients. This condition may result in increased serotonin reuptake and shorter serotonergic activity in male patients with OSA.[88] On the other hand, the presence of the L allele results in shorter serotonin activity because of the relatively faster reuptake of 5-HT. The L/L genotype was more frequent in male patients than in female patients. This genotype difference between the sexes may also be associated with serotonin depletion in male patients. On the basis of these genotype differences, serotonin depletion caused by fast 5-HT reuptake appears to predispose male patients to OSA. The L/S genotype was more frequent in male OSA patients than in male controls.[88] This genotype results in a moderate serotonin reuptake activity and appears to be associated with the occurrence of OSA in male patients. It was suggested that the S allele of the 5HTTLPR may identify patients at risk for developing insomnia with fluoxetine (a serotonin reuptake inhibitor drug) treatment. This is also an indirect support of the finding of this study that the presence of S allele is protective against OSA.[88] Despite the fact that functional results of VNTR polymorphism are unclear, the genotype differences found in this study suggest that polymorphism of 5-HT transporter gene may be associated with OSA.

The use of serotonergic antidepressants may cause sleep disorder.[94] However, many of the drugs tested to evaluate the effects of 5-HT receptor antagonists have not produced significant improvement in sleep apnoea.[95] Serotonin receptor subtypes may affect efficiency of the 5-HT receptor antagonists. Systemic administration of serotonin 2A and 2C receptor agonists were shown to improve upper airway collapsibility, at least in rats.[96] It is possible that the serotonergic activity is shorter in male patients because of STG polymorphism. Further studies are necessary to discover the affect of serotonergic antagonists in male OSA patients.

OSA in children and leukotriene receptor genes

Cysteinyl leukotriene receptors 1 and 2 (LT1-R and LT2-R) are expressed in human tonsils, and are deeply involved in inflammatory and allergic responses.[97] Tissue damage through inflammation during respiratory infection or through recurrent vibratory trauma will in turn promote the development of an inflammatory response. This could lead to mucosal swelling and subsequently to upper airway obstruction, suggesting that LT1-R and LT2-R gene upregulation may underlie components of the pathophysiological mechanisms linking the enlargement of the tonsillar tissue to the emergence of sleep apnoea in snoring children. This may possibly occur through enhanced upper airway inflammation linked to mechanical irritation of the upper airway mucosa due to snoring.[98] Therefore, upregulation of LT-R expression would occur, leading ultimately to accelerated growth of the tonsillar tissue and to upper airway obstruction during sleep.

The rapidly accumulating body of evidence in adult OSA patients lends credible support to the theory that the recurrent vibration of the air column in the upper airway due to snoring will induce mechanical trauma. The study showed no expression of these receptors in cells located in germinal centres of the tonsillar lymphoid tissue and one possible explanation is that these cells have migrated from the vasculature to occupy their sites within the tonsils.[99] LT1-R antagonists such as montelukast have found their application in the treatment of asthma and allergic rhinitis in children, while no antagonist for the LT2-R has been developed thus far. The use of LT-R antagonists may be a potential future therapeutic consideration in treating children with OSA. This has been shown by a recent study that demonstrated improved breathing during sleep in children with sleep-disordered breathing after oral therapy with montelukast.[100]

OSA and other associations

OSA has been linked with various loci of the major histocompatibility complex (MHC) complex. A study showed a twofold increase in the HLA-A2 antigen.[101] HLA-A2 positive subjects with OSA were more obese than OSA patients negative for this antigen, suggesting a relationship between this genetic marker and obesity. Another study[102] implicated HLA-A33, HLA-DRB1*03, DQB1*02 with OSA and HLA-B7, B65, B63, B73 with primary snoring, although the significance was not consistent.

An increased frequency for the Lewis blood group phenotype Le (a+b-) is also seen in snorers compared with non-snorers, although the implications of this finding have not yet been clarified.

Also conflicting are the findings regarding the possible link between apolipoprotein E genotype 4, and OSA. Apolipoprotein E is a polymorphic protein arising from three alleles at a single gene locus on chromosome 19q13. Although no difference was found in the apolipoprotein E levels between OSA patients and controls, a higher proportion of homozygotes for the E4 genotype was observed in the sleep apnoea group, although the finding was not statistically significant.[103] Another study showed that the risk for AHI >15 was doubled among homozygotes, independently of sex and BMI.[104] In a third study no association was found.[105] A recent paper showed that there is a disease susceptibility locus for obstructive sleep apnoea in the region of ApoE (chromosome 19), but ApoE itself is unlikely to be the causative locus.[106]

A study reported an association between angiotensin-converting enzyme (ACE) gene polymorphism and severity of sleep apnoea, something that shows a potential link between this gene and severity of OSA.[107] Another study compared ACE activity in patients with OSA and control subjects and showed that ACE activity is increased in patients with OSA, a finding independent of the presence of arterial hypertension, but the distribution of ACE genotypes and of allelic frequency in OSA patients did

not differ from that determined in healthy subjects.[108] Therefore, although the increased ACE activity would seriously jeopardise the endothelial function and vascular structure, increasing the prevalence for cardiovascular events, no significant correlation was found between AHI and ACE activity in OSA patients. A more recent study found no association between ACE and OSA.[109]

Elevations in various factors have been found in serum from patients with OSA, and could also serve as biological markers. Increased levels of circulating endothelin-1,[110,111] a peptide with vasoconstrictor effects, have been demonstrated in OSA subjects as compared with control subjects and its levels declined after therapy with CPAP. Another study, however, showed that plasma endothelin-1 precursor but not endothelin-1 levels are elevated and decline after therapy with nasal CPAP.[112] The inflammatory cytokine, tumour necrosis factor (TNF)-α, has also been shown to be elevated in OSA patients when compared to controls.[113,114] Plasma fibrinogen concentration and whole blood viscosity have been reported to be higher in the morning than afternoon in a small number of untreated OSA patients, with no such diurnal change in OSA patients treated with CPAP.[115] Also, variations in the levels of heat shock proteins, proteins thought to respond to stresses such as hypoxia, have been examined in small numbers of OSA patients, with results that were ambivalent.[116]

CONCLUSION

OSA is a multifactorial entity and only recently the complex genetical and environmental links begun to be elucidated. Racial studies and chromosomal mapping, familial studies and twin studies have provided evidence for the possible link between the OSA phenotypes and genetic loci that could prove to be markers for further research, including obesity, fat distribution, snoring, and sleep regulation. Also, the potential role of serotonin and the regulation of upper airway tone during sleep could prove a field of pharmaceutical intervention. Tissue damage, through recurrent vibratory trauma induced by snoring, and the role of inflammatory mediators could also be a target for drug therapy. On the other hand, if their exact significance is clarified, the importance of the increased levels of certain molecules, such as TNF-α, ET-1 and plasminogen, could give physicians a quantitative tool, apart from the AHI index, for determining the severity of OSA and the risk for adverse cardiovascular effects, making it possible for a scoring system to be developed.

ACKNOWLEDGEMENTS

The authors would like to thank Dimosthenis Lykouras for his assistance in editing.

REFERENCES

1. Guilleminault C. Clinical features and evaluation of obstructive sleep apnea. In: Kryeger M, Roth T, Dement WC (eds). Principles and practice of sleep medicine. 2nd ed. Philadelphia: WB Saunders, 1994.
2. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep disordered breathing among middle-aged adults. N Engl J Med 1993;328:1230-5.
3. Rosen CL, Larkin EK, Kirchner HL, et al. Prevalence and risk factors for sleep-disordered breathing in 8- to 11-year-old children: association with race and prematurity. J Pediatr 2003;142:383-9.
4. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002;165:1217-39.
5. Punjabi NM, Sorkin JD, Katzel LI, Goldberg AP, Schwartz AR, Smith PL. Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. Am J Respir Crit Care Med 2002;165:677-82.
6. Leung RS, Bradley TD. Sleep apnea and cardiovascular disease. Am J Respir Crit Care Med 2001;164:2147-65.
7. Newman AB, Nieto FJ, Guidry U, et al. Relation of sleep-disordered breathing to cardiovascular disease risk factors: the Sleep Heart Health Study. Am J Epidemiol 2001;154:50-9.
8. Flemons WW, Buysse D, Redline S, et al. Sleep-related breathing disorders in adults: recommendations and measurement techniques in clinical research. Report of an American Academy of Sleep Medicine Task Force. Sleep 1999;22:533-70.
9. Taheri S, Mignot E. The genetics of sleep disorders. Lancet Neurology 2002;1:242- 50.
10. Redline S, Tishler P. The genetics of sleep apnea. Sleep Medicine Reviews 2003;4:583-602.
11. Redline S, Tosteson T, Boucher MA, Millman RP. Measurement of sleep-related breathing disturbance in epidemiologic studies: Assessment of the validity and reproducibility of a portable monitoring device. Chest 1991;100:1281-6.
12. Strohl KP, Saunders NA, Feldman NT, Hallett M. Obstructive sleep apnea in family members. N Engl J Med 1978;299:969-73.
13. Redline S, Tishler PV, Tosteson TD, et al. The familial aggregation of obstructive sleep apnea. Am J Respir Crit Care Med 1995;151:682-7.
14. Mathur R, Douglas NJ. Family studies in patients with the sleep apnea-hypopnea syndrome. Ann Intern Med 1995;122:174-8.
15. Gislason T, Johannsson JH, Haraldsson A, et al. Familial predisposition and cosegregation analysis of adult obstructive sleep apnea and the sudden infant death syndrome. Am J Respir Crit Care Med 2002;166:833-8.
16. El-Bayadi S, Millman RP, Tishler PV, Rosenberg C, Boucher MA, Redline S. A family study of sleep apnea: anatomic and physiologic interactions. Chest 1990;98: 554–9.
17. Kaprio J, Koskenvuo M, Partinen M, Telakivi I. A twin study of snoring. Sleep Res 1988;17:365.
18. Carmelli D, Bliwise DL, Swan GE, Reed T. Genetic factors in self-reported snoring and excessive daytime sleepiness: a twin study. Am J Respir Crit Care Med 2001;164: 949-52.
19. Redline S, Tosteson T, Tishler PV, Carskadon MA, Millman RP. Familial aggregation of symptoms associated with sleep-related breathing disorders. Am Rev Respir Dis 1992;145:440-4.
20. Jennum P, Hein HO, Suadicani P, Sorensen H, Gyntelberg F. Snoring, family history, and genetic markers in men: The Copenhagen Male Study. Chest 1995;107:1289-93.
21. Guilleminault C, Partinen M, Hollman K, Powell N, Stoohs R. Familial aggregates in obstructive sleep apnea syndrome. Chest 1995;107:1545-51.
22. Pillar G, Lavie P. Assessment of the role of inheritance in sleep apnea syndrome. Am J Respir Crit Care Med 1995;151:688-91.
23. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 1997;27:325-51.

24. Bouchard C. Genetic determinants of regional fat distribution. Hum Reprod 1997;12(suppl 1):1-5.

25. Tishler PV, Redline S, Ferrette V, Hans MG, Altose MD. The association of sudden unexpected infant death with obstructive sleep apnea. Am J Respir Crit Care Med 1996;153:1857-63.

26. Palmer LJ, Buxbaum SG, Larkin EK, et al. Whole Genome Scan for obstructive sleep apnea and obesity in African-American Families. Am J Respir Crit Care Med 2004;169:1314-21.

27. Redline S, Tishler PV, Hans MG, Tosteson TD, Strohl KP, Spry K. Racial differences in sleep-disordered breathing in African-Americans and Caucasians. Am J Respir Care Med 1997;155:186-92.

28. Baldwin M, Kolbe J, Troy K, et al. Racial differences in severity of sleep apnea between Maori, Pacific Islanders and Europeans [abstract]. Am J Respir Crit Care Med 1996;153:A357.

29. Luke A, Guo X, Adeyemo AA, et al. Heritability of obesity-elated traits among Nigerians, Jamaicans, and US black people. Int J Obes Relat Metab Disord 2001;25:1034-41.

30. Sanders MH, Redline S. Obstructive sleep apnea/hypopnea syndrome. Curr Treat Options Neurol 1999;4:279-90.

31. Redline S, Tishler PV, Schuchter M, Aylor J, Clark K, Graham G. Risk factors for sleep Disordered breathing in children. Associations with obesity, race, and respiratory problems. Am J Respir Crit Care Med 1999;159:1527-32.

32. Smith PL, Gold AR, Meyers DA, Haponik EF, Bleeker ER. Weight loss in mild to moderately obese patients with obstructive sleep apnea. Ann Int Med 1985;103:850-5.

33. Mortimore IL, Marshall I, Wraith PK, Sellar RJ, Douglas NJ. Neck and total body fat distribution in nonobese and obese patients with sleep apnea compared to that in control subjects. Am J Respir Crit Care Med 1998,157:280-4.

34. Wilcox I, McNamara SG, Collins FL, Grunstein RR, Sullivan CE. "Syndrome Z": the interaction of sleep apnea, vascular risk factors and heart disease. Thorax 1998;53(suppl 3):S25-8.

35. Reaven GM. Diet and syndrome X. Curr Atheroscler Rep 2000;2:503-7.

36. Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Sleep apnea and daytime sleepiness and fatigue: relation to physical obesity, insulin resistance, and hypercytokinemia. J Clin Endocrinol Metab 2000;85:1151-8.

37. Comuzzie A, Hixson J, Almasy L, et al. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat Genet 1997;15:273-6.

38. Sina M, Hinney A, Ziegler A, et al. Phenotypes in three pedigrees with autosomal dominant obesity caused by haploinsufficiency mutations in melanocortin-4 receptor gene. Am J Hum Genet 1999;65:1501-7.

39. Bray G, Bouchard C. Genetics of human obesity: research directions. FASEB J 1997;11:937-45.

40. Wiesner G, Vaz M, Collier G, et al. Leptin is released from the human brain: influence of adiposity and gender. J Clin Endocrinol Metab 1999 ;84:2270-4.

41. O'Donnell CP, Schaub CD, Haines AS, et al. Leptin prevents respiratory depression in obesity. Am J Respir Crit Care Med 1999;159:1477-84.

42. Sinton CM, Fitch TE, Gershenfeld HK. The effects of leptin on REM sleep and slow wave delta in rats are reversed by food deprivation. J Sleep Res 1999;8:197-203.

43. Lowe AA, Ozbek MM, Miyamoto K, Pae EK, Fleetham JA. Cephalometric and demographic characteristics of obstructive sleep apnea: an evaluation with partial least squares analysis. Angle Orthod 1997;67:143-53.

44. Bacon WH, Krieger J, Turlot J-C, Stierle JL. Craniofacial characteristics in patients with obstructive sleep apnea syndrome. Cleft Palate J 1988;25:374-8.

45. Guilleminault C, Riley R, Powell N. Obstructive sleep apnea and abnormal cephalometric measurements. Implications for treatment. Chest 1984;86:793-4.

46. Sanford LP, Ormsby I, Gittenberger-de Groot AC, et al. TGF Beta-2 knockout mice have multiple developmental defects that are nonoverlapping with other TGF Beta knockout phenotypes. Development 1997;124:2659-70.

47. Kurihara Y, Kurihara H, Suzuki H, et al. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature 1994;368:703-10.

48. Lohnes D, Mark M, Mendelsohn C, et al. Function of the retinoic acid receptors (RARs) during development. (I) Craniofacial and skeletal abnormalities in RAR double mutants. Development 1994;120:2723-48.

49. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 1990;323:152-9.

50. Thompson MD, Comings DE, Abu-Ghalazah R, et al. Variants of the orexin 2/hcrt2 receptor gene identified in patients with excessive daytime sleepiness and patients with Tourette's syndrome comorbidity. Am J Med Neuropsychiatr Genet 2004;129:69-75.

51. Cherniack NS. Respiratory dysrhythmias during sleep. N Engl J Med 1981;305:324-30.

52. Dempsey JS, Skatrud JB. A sleep-induced apneic threshold and its consequences. Am Rev Respir Dis 1986;133:1163-70.

53. Kawakami Y, Yamamoto H, Yoshikawa T, Shida A. Chemical and behavioral control of breathing in twins. Am Rev Respir Dis 1984;129:703-7.

54. Thomas DA, Swaminathan S, Beardsmore CS, et al. Comparison of peripheral chemoreceptor responses in monozygotic and dizygotic twin infants. Am Rev Respir Dis 1993;148:1605-9.

55. Redline S, Leitner J, Arnold J, Tishler P, Altose M. Ventilatory control abnormalities in familial sleep apnea. Am J Respir Crit Care Med 1997;56:155-60.

56. Pillar G, Schnall RP, Peled N, Oliven A, Lavie P. Impaired respiratory response to resistive loading during sleep in healthy offspring of healthy patients with obstructive sleep apnea. Am J Respir Crit Care Med 1997;155:1602-8.

57. Paton J, Swaminathan S, Sargent C, Keens T. Hypoxic and hypercapnic ventilatory responses in awake children with congenital central hypoventilation syndrome. Am Rev Respir Dis 1989;140:368-72.

58. Sakai T, Wakizaka A, Matsuda H, Nirasawa Y, Yasuo I. Point mutation in exon 12 of the receptor tyrosine kinase proto-oncogene RET in Ondine – Hirschsprung syndrome. Pediatrics 1998;101:924-26.

59. Amiel J, Salomon R, Attie T, et al. Mutations of the RET-GDNF signaling pathway in Ondine's curse [Letter]. Am J Hum Gen 1998;62:715-7.

60. Croaker GD, Shi E, Simpson E, et al. Congenital central hypoventilation syndrome and Hirschsprung's disease. Arch Dis Child 1998;78:316-22.

61. Durbec P, Marcos-Gutierrez CV, Kilkenny C, et al. GDNF signalling through the Ret receptor tyrosine kinase. Nature 1996;381:789-93.

62. Amiel J, Laudier B, Attie-Bitach T, et al. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat Genet 2003;33:459-61 (and appendix).

63. Weese-Mayer DE, Berry-Kravis EM, Zhou L, et al. Idiopathic congenital central hypoventilation syndrome: Analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. Am J Med Genet 2003;123A:267-78.

64. Romeo G, Ronchetto P, Luo Y, et al. Point mutations affecting the tyrosine kinase domain of the RET protooncogene in Hirschsprung's disease. Nature 1994;367:377-8.

65. Bolk S, Angrist M, Schwartz S, Silvestri J, Weese-Mayer D, Chakravarti A. Congenital central hypoventilation syndrome: mutation analysis of the receptor tyrosine kinase RET. Am J Med Genet 1996;63:603-9.

66. Burton MD, Kawashima A, Brayer JA, et al. RET proto-oncogene is important for the development of respiratory CO_2 sensitivity. J Auton Nervous Sys 1997;63:137-43.

67. Kuwaki T, Cao W-H, Kurihara Y, et al. Impaired ventilatory responses to hypoxia and hypercapnia in mutant mice deficient in endothelin-1. Am J Physiol 1996;270:R1279-86.

68. Jacquin TD. Reorganization of pontine rhythmogenic neuronal networks in Krox-20 knockout mice. Neuron 1996;17:747-58.

69. Erickson JT, Conover JC, Borday V, et al. Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J Neurosci 1996;16:5361-71.

70. Tankersley CG, Fitzgerald RS, Levitt RC, Mitzner WA, Ewart SL, Kleeberger SR. Genetic control of differential baseline breathing pattern. J Appl Physiol 1997;82:874-81.

71. Scheuermann BW, Kowalchuk JM, Paterson DH, Cunningham DA. Peripheral chemoreceptor function after carbonic anhydrase inhibition during moderate-intensity exercise. J Appl Physiol 1999;86:1544-51.

72. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000;6:991-7.

73. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27:469-74.

74. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999;98:437-51.

75. Mignot E, Lammers GJ, Ripley B, et al. The role of CSF hypocretin measurement in the diagnosis of narcolepsy and hypersomnia. Arch Neurol (in press).

76. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98:365-76.

77. Douse MA, White DP. Serotonergic effects on hypoglossal neural activity and reflex responses. Brain Res 1996;726:213-22.

78. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000;355:39-40.

79. Bayliss DA, Viana F, Talley EM, Berger AJ. Neuromodulation of hypoglossal motoneurons: cellular and developmental mechanisms. Respir Physiol 1997;110:139-50.

80. Ribeiro-do-Valle LE, Metzler CW, Jacobs BL. Facilitation of masseter EMG and masseteric (jaw-closure) reflex by serotonin in behaving cats. Brain Res 1991;550:197-204.

81. McGinty DJ, Harper RM. Dorsal raphe neurons: depression of firing during sleep in cats. Brain Res 1976;101:569-75.

82. Jelev A, Sood S, Liu H, Nolan P, Horner RL. Microdialysis perfusion of 5-HT into hypoglossal motor nucleus differentially modulates genioglossus activity across natural sleep-wake state in rats. J Physiol 2001;532:467-81.

83. Heym J, Steinfels GF, Jacobs BL. Activity of serotonin-containing neurons in the nucleus raphe pallidus of freely moving cats. Brain Res 1982;251:259-76.

84. Van Lunteren E. Muscles of the pharynx: structural and contractile properties. Ear Nose Throat J 1993;72:27-29,33.

85. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083-152.

86. Fenik P, Veasey SC. Pharmacological characterization of serotonergic receptor activity in the hypoglossal nucleus. Am J Respir Crit Care Med 2003;167:563-9.

87. Sakai K, Toshinori T, Nakayama H, et al. Serotonin-2A and 2C receptor gene polymorphisms in Japanese patients with obstructive sleep apnea. Int Med 2005;44:928-33.

88. Yilmaz M, Bayazit AY, Ciftci TU, et al. Association of serotonin transporter gene polymorphism with obstructive sleep apnea syndrome. The Laryngoscope 2005;115:832-6.

89. Offenbaecher M, Bondy B, de Jonge S, et al. Possible association of fibromyalgia with a polymorphism in the serotonin transporter gene regulatory region. Arthritis Rheum 1999;42:2482-8.

90. Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621-4.

91. Serreti A, Catalano M, Smeraldi E. Serotonin transporter gene is not associated with symptomatology of schizophrenia. Schizophr Res 1999;35:33-9.

92. Nieto F, Young TB, Lind BK, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community based study. Sleep Heart Health Study. JAMA 2000;283:1829-36.

93. Pillar G. Malhotra A, Fogel R, et al. Airway mechanics and ventilation in response to resistive loading during NREM sleep: The influence of gender. Am J Respir Crit Care Med 2000;162:1627-32.

94. Winkelman JW, James L. Serotonergic antidepressants are associated with REM sleep without atonia. Sleep 2004;27:317-21.

95. Veasey SC. Serotonin agonists and antagonists in obstructive sleep apnea: therapeutic potential. Am J Respir Med 2003;2:21-9.

96. Ogasa T, Ray AD, Michlin CP, et al. Systemic administration of serotonic 2A/2C agonist improves upper airway stability in Zucker rats. Am J Respir Crit Care Med 2004; 170:804-10.

97. Goldbart AD, Goldman JL, Li RC, Brittian KR, Tauman R, Gozal D. Differential Expression of Cysteinyl Leukotriene Receptors 1 and 2 in Tonsils of Children With Obstructive Sleep Apnea Syndrome or Recurrent Infection. Chest 2004;126:13-8.

98. Ebenfelt A, Ivarsson M. Neutrophil migration in tonsils. J Anat 2001;198:497-500.

99. Yoshizawa T, Kurashina K, Sasaki I, et al. Analysis of HLA antigens in patients with obstructive sleep apnea syndrome [Abstract]. Am Rev Respir Dis 1991;143(suppl):A381.

100. Brunetti L, Francavilla R, Miniello VL, et al. Influence of HLA antigens and OSAS in childhood: a preliminary report. J Sleep Res 2005;14:157-62.

101. Saarelainen S, Lehtimaki T, Kallonene E, Laasonein K, Poussa T, Neiminen MM. No relation between apolipoprotein E alleles and obstructive sleep apnea. Clin Genet 1998;53:147-8.

102. Kadotani H, Kadotani T, Young T, et al. Association between apolipoprotein E, epsilon4 and sleep disordered breathing in adults. JAMA 2001;285:2888-90.

103. Foley DJ, Masaki K, White L, Redline S. Relationship between lipoprotein E, epsilon 4 and sleep disordered breathing at different ages. JAMA 2001;286:1447-8.

104. Xiao Y, Huang X, Qiu C, Qhu X, Liu Y. Angiotensin 1-converting enzyme gene polymorphism in Chinese patients with obstructive sleep apnea syndrome. Chinese Med J 1999;112:701-4.

105. Barcelo A, Elorza MA, Barbe F, Santos C, Mayoralas LR, Agusti AGN. Angiotensin converting enzyme in patients with sleep apnea syndrome: plasma activity and gene polymorphisms. Eur Respir J 2001;17:728-32.

106. Saarelainen S, Seppala E, Laasonen K, Hasan J. Circulating endothelin-1 in obstructive sleep apnea. Endothelium 1997;5:115-8.

107. Phillips BG, Narkiewicz K, Pesek CA, et al. Effects of obstructive sleep apnea on endothelin-1 and blood pressure. Journal of Hypertension 1999;17:61-6.

108. Jordan W, Reinbacher A, Cohrs S, et al. Obstructive sleep apnea: Plasma endothelin-1 precursor but not endothelin-1 levels are elevated and decline with nasal continuous positive airway pressure. Peptides 2005;1654-60.

109. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997;82:1313-6.

110. Entzian P, Linnemann K, Schlaak M, Zabel P. Obstructive sleep apnea syndrome and circadian rhythms of hormones and cytokines. Am J Respir Crit Care Med 1996;153:1080-6.

111. Chin K, Ohi M, Kita H, et al. Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 1996;153:1972-6.

112. Noguchi T, Chin K, Ohi M, et al. Heat shock protein 72 level decreases during sleep in patients with obstructive sleep apnea syndrome. Am J Respir Crit Care Med 1997;155:1316-22.

113. Goldbart AD, Goldman JL, Veling MC, Gozal D. Leukotriene modifier therapy for mild sleep-disordered breathing in children. Am J Respir Crit Care Med 2005;172:364-70.

114. Larkin EK, Patel SR, Redline S, Mignot E, Elston RC, Hallmayer J. Apolipoprotein E and obstructive sleep apnea: evaluating whether a candidate gene explains a linkage peak. Genet Epidemiol 2006;30:101-10.

115. Lin L, Finn L, Zhang J, Young T, Mignot E. Angiotensin-converting enzyme, sleep disordered breathing, and hypertension. Am J Respir Crit Care Med 2004;170:1349-53.

116. De Geus EJC, Posthuma D, Kupper N, et al. A whole genome scan for 24-hour respiration rate: A major locus at 10q26 influences respiration during sleep. Am J Hum Genet 2005;76:100-11.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Preoperative pharmacological management of phaeochromocytoma

A.N.A. van der Horst-Schrivers*, M.N. Kerstens, B.H.R. Wolffenbuttel

Department of Endocrinology, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands, *corresponding author: tel.: +31 (0)50-361 61 61, e-mail: a.n.a.van.der.horst@int.umcg.nl

ABSTRACT

Phaeochromocytoma is a rare catecholamine-secreting neuroendocrine tumour with a high cardiovascular morbidity and mortality if left untreated. Surgical resection is the only curative therapy. During surgery there is a high risk of massive release of catecholamines, which can result in potentially fatal hypertensive crises and cardiac arrhythmias. Administration of vasoactive drugs such as (non)selective α- and β-antagonists and calcium channel blocking agents have reduced the operation risk. Guidelines for the preoperative medical management of the patient with a phaeochromocytoma are mainly based on retrospective studies and case reports. We reviewed the relevant literature on this subject. In addition, we compared the several preoperative treatment protocols of the eight university medical centres in the Netherlands.

KEYWORDS

Phaeochromocytoma, preoperative management

INTRODUCTION

Phaeochromocytoma is a rare catecholamine-secreting neuroendocrine tumour, with an estimated prevalence among hypertensive patients of 0.1 to 0.6%. Approximately 90% of phaeochromocytomas arise in the adrenal gland, and about 10% originate from extra-adrenal chromaffin tissue.[1] In hereditary cancer syndromes such as multiple endocrine neoplasia type 2 and von Hippel-Lindau syndrome, phaeochromocytoma can occur bilaterally in both adrenal glands.[2] Symptoms and signs of a phaeochomocytoma result from an uncontrolled release of catecholamines (norepinephrine, epinephrine, dopamine). Catecholamines are agonists of α- and β-adrenoceptors (*table 1*). Most patients suffer from hypertension, which can be paroxysmal or sustained.[3] The classic triad of complaints consists of headache, perspiration and palpitations. Other clinical findings can be orthostatic hypotension, tremor, pallor, panic attacks, visual disturbances and weight loss.[4] A phaeochromocytoma is a potentially life-threatening disease with a highly increased risk for cardiovascular complications as myocardial infarction, coronary spasms, arrhythmias, cardiomyopathy, stroke and pulmonary oedema.[3,5-8] Surgical resection is the only curative therapy. The first successful operation was performed by Roux in 1926.[9] Initially, surgical resection of a phaeochromocytoma was faced with a high perioperative mortality rate of 20 to 45%.[10] This mortality rate dropped to 0 to 2.9% during the second half of the last century.[11] Important developments which have contributed to this major reduction in perioperative mortality rate are better imaging techniques for accurate preoperative tumour localisation, improvements in surgical and anaesthetic techniques, and better preoperative medical management.

Table 1. *Catecholamine effects on α- and β-adrenoceptors*

Receptor	Effect	Catecholamine
α_1	Vasoconstriction (venous & arterial), intestinal relaxation, stimulation of glycogenolysis, uterus contraction, mydriasis	Norepinephrine
α_2	Inhibition norepinephrine release (presynaptic), vasoconstriction, lowering insulin release, sedation	Epinephrine and norepinephrine
β_1	Positive chronotropy, positive dromotropy & positive inotropy, stimulation renin release, lipolysis	Epinephrine and norepinephrine
β_2	Vasodilatation (muscle), bronchodilatation	Epinephrine

We reviewed the relevant literature on the preoperative pharmacological management of a phaeochromocytoma. Original studies written in English and published between 1960 and 2005 were retrieved by a search in PubMed using the following MESH headings and/or text words: phaeochromocytoma, preoperative management or surgery and phenoxybenzamine or doxazosin or prazosin or calcium channel blockers. We also used the references of the articles found. We included only articles with sufficient information on the outcome of one or more preoperative regimens. For this article we identified 26 articles, one prospective study, seven case or patient series and the remaining retrospective studies.

PHAEOCHROMOCYTOMA AND PERIOPERATIVE RISKS

Surgery in itself carries a very high risk of evoking a massive release of catecholamines into the circulation, resulting in one or more of the serious cardiovascular complications mentioned previously. Induction of anaesthesia, intubation, first incision, creation of a pneumoperitoneum and tumour manipulation are critical moments for such an uncontrolled catecholamine release.[12-14] Postoperatively, the sudden drop in catecholamine levels may result in hypotension and hypoglycaemia. Catecholamines inhibit insulin secretion and stimulate glycogenolysis and lipolysis. Normalisation of the excessive catecholamine secretion after tumour resection in the presence of empty glycogen storages can lead to a 'rebound' hyperinsulinaemia, thus resulting in hypoglycaemia. There is a positive correlation between the perioperative complication rate and the size of the tumour, length of the operation and the serum levels of catecholamines.[15]

PREOPERATIVE PHARMACOLOGICAL MANAGEMENT

The goals of preoperative pharmacological treatment are optimal control of hypertension and of the other phaeochromocytoma-related symptoms, and prevention of perioperative complications. Several drugs have been recommended for this purpose, including selective and non-selective α- and β-adrenoceptor antagonists, calcium channel blockers, and drugs that inhibit catecholamine synthesis. However, there are no randomised controlled trials addressing the optimal medical treatment of a phaeochromocytoma, and it is doubtful whether these will ever be performed in view of the low prevalence of this disorder. Therefore, the best available evidence is derived from retrospective studies, patient series and case reports. According to some studies, preoperative treatment with adrenoceptor blocking agents is not obligatory.[16,17] No difference in perioperative mortality or morbidity was found in a retrospective study comparing 31 patients receiving adrenoceptor antagonists preoperatively with 29 patients who did not, although intraoperative blood pressure rises were more pronounced in the latter group.[18] It should be noted, however, that α- and β-antagonists were administered during surgery in both groups if indicated. Besides preoperative pharmacological treatment, preoperative restoration of the circulating volume is also recommended by most authorities. The prevailing hypothesis is that the patient with a phaeochromocytoma has a reduced intravascular volume as a result of catecholamine-mediated vasoconstriction.[19,20] Administration of α-antagonists results in vasodilatation, leading to intravascular volume depletion. This needs to be compensated for by a high sodium diet or a saline infusion.

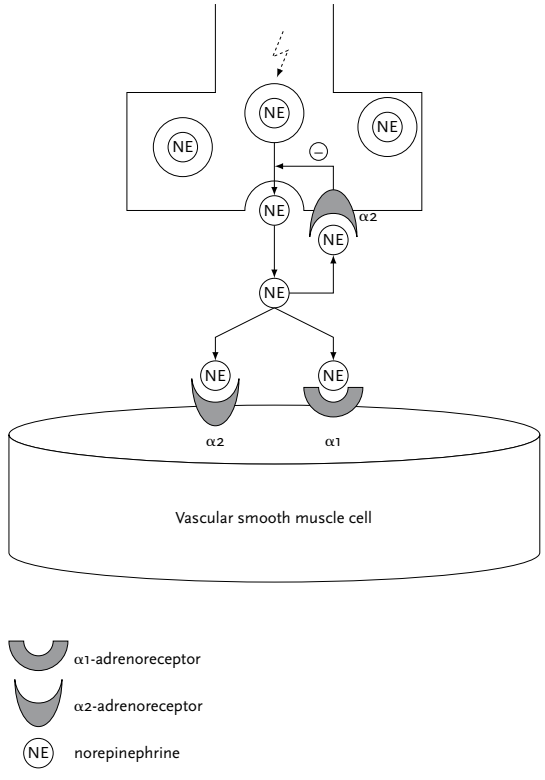
NONSELECTIVE α-ANTAGONISTS

Since the early 1950s, phenoxybenzamine has been widely used as the main drug for preoperative management of a phaeochromocytoma. It is a noncompetitive α_1- and α_2-antagonist, with a maximal effect four to six hours after administration and a pharmacological half-life of 24 hours. A regular starting dose is 10 mg twice daily, which can be increased to a daily dose of 80 to 100 mg/day (maximum 1 mg/kg). To ensure adequate blood pressure control, it is recommended that the blood pressure should not be higher than 160/90 mmHg, with orthostatic hypotension not exceeding 80/45 mmHg.[5] Disadvantages of phenoxybenzamine are the occurrence of reflex tachycardia and excessive orthostatic hypotension. Reflex tachycardia is caused by blockade of α_2-receptors localised in the presynaptic membrane. Stimulation of the α_2-receptor inhibits norepinephrine release. Therefore, α_2-receptor blockade will interrupt this negative feedback mechanism, eliciting undesirable chronotropic and inotropic effects (*figure 1*). Other disadvantages of phenoxybenzamine are central sedation and prolonged duration of action. Continuing α-receptor antagonism in combination with the postoperative decrease in catecholamine levels can result in prolonged hypotension after surgery. Despite these disadvantages, several retrospective studies suggest that preoperative treatment with phenoxybenzamine has resulted in a significant reduction in operation mortality.[21-27] The need for intraoperative α_2-receptor blockade (phentolamine) is also reduced in pretreated patients.[25] It is probably sufficient to start phenoxybenzamine four to seven days preoperatively, as no clinically relevant benefit has been demonstrated with a longer preoperative treatment period.[13,21]

SELECTIVE α_1-ANTAGONISTS

Theoretically, selective α_1-antagonists offer several advantages when compared with phenoxybenzamine. These drugs do not elicit reflex tachycardia, in the absence of α_2-receptor affinity (*figure 1*). In addition, these drugs have a relatively short duration of action as a result of their competitive inhibition. Consequently, adequate dose titration could be achieved more quickly and the risk period for postoperative hypotension could be shortened. The α_1-antagonists doxazosin and prazosin are registered in the Netherlands. Doxazosin has a half-life of 16 to 30 hours and can be administered in a single dose varying between 1 to 16 mg. Prazosin has a much shorter half-life of two to three hours and needs to be ingested three to four times daily. In a retrospective study including 35 patients with phaeochromocytoma, eight patients were treated with phenoxybenzamine (20-120 mg per day) and 27 patients with doxazosin (2-16 mg per day).[28] All patients in the phenoxybenzamine group received a β-blocker as standard treatment *vs* only nine patients in the doxazosin group. Administration of doxazosin was accompanied by a lower preoperative diastolic blood pressure (78 *vs* 92 mmHg) and a lower intraoperative heart rate (78 *vs* 94 beats/min) during tumour manipulation. The patients receiving doxazosin required more phentolamine intraoperatively, although this difference (11.1 *vs* 9.6 mg) was not statistically significant. However, they required less labetalol (15.8 *vs* 33.1 mg, p=0.080). In addition, postoperative recovery seemed to be better with doxazosin, as reflected by a higher blood pressure (116/64 *vs* 100/55 mmHg) and a lower demand for intravenous fluids with less oedema. Mortality rate, however, was not different between the two groups.[28] Another retrospective study was not able to demonstrate a difference between phenoxybenzamine, doxazosin and prazosin with respect to blood pressure control and amount of postoperative fluid replacement.[29] In yet another report with only four patients, adequate blood pressure control was not achieved with prazosin, which even resulted in postponement of surgery in one patient.[30]

Figure 1. *Mechanism of action of α_1- and α_2-adrenoreceptor antagonists*



BETA-ANTAGONISTS

The main goal of preoperative β-receptor blockade is prevention of cardiac arrhythmias.[22,31,32] Propranolol, atenolol and metoprolol are commonly used for this purpose. Administration of a β-antagonist is absolutely contraindicated in the absence of effective α-receptor blockade, as unopposed stimulation of α-receptor mediated vasoconstriction and loss of the β-receptor mediated vasodilatation may cause a dangerous rise in blood pressure.[33] This has also been described with the use of the combined α- and β-antagonist labetalol, which demonstrates a relatively stronger antagonism towards the α-receptor.[34] Caution is warranted when administering β-antagonists to patients with severe left ventricular dysfunction, a condition which is not uncommon with a phaeochromocytoma due to cardiomyopathy induced by chronic exposure to high catecholamine levels.[3] Preoperative treatment with β-antagonists is likewise predominantly based on pathophysiological considerations and retrospective studies. Beta-antagonist sometimes fail in preventing cardiac arrhythmias, and these drugs are not indicated in every patient.[3] Orchard *et al.* describe 108 patients with a phaeochromocytoma, 95 of whom received an α- and β-antagonist. Of these, five patients developed a cardiac arrhythmia, despite the fact that they all were on β-antagonists.[21] In another retrospective study, patients were pretreated with phenoxybenzamine alone or with the combination of phenoxybenzamine and propranolol. The frequency of cardiac arrhythmias during surgery was not found to be different between individuals who were treated preoperatively with propranolol and those who were not.[36]

CALCIUM CHANNEL BLOCKERS

Calcium channel blockers cause smooth muscle relaxation in peripheral and coronary arteries through inhibition of the epinephrine-stimulated calcium influx. Consequently, peripheral vascular resistance is reduced and catecholamine-induced spasms of coronary arteries might be prevented.[37] As with β-antagonists some calcium channel blockers have to be used carefully in patients with left ventricular dysfunction. Although it has been found that calcium channel blockers influence the release of catecholamines *in vitro*, this has not been demonstrated *in vivo*.[38-40] Calcium channel blockers can also be given intravenously during surgery. There are a few studies describing the effect of calcium channel blockers administered before or during surgery. Proye *et al*. reported good results with intraoperative use of nicardipine. They measured a 42% reduction of systemic vascular resistance and achieved an effective blood pressure control, despite a significant rise in catecholamine levels during tumour manipulation.[37] In another study, 29 out of 113 phaeochromocytoma patients undergoing surgery were successfully treated with a calcium channel blocker.[17] Lebuffe *et al*. recently described a group of 105 patients who had been treated preoperatively with a calcium channel blocker. Blood pressure was well controlled intraoperatively, and the perioperative mortality rate in this retrospective series was relatively low at 2.8%.[41]

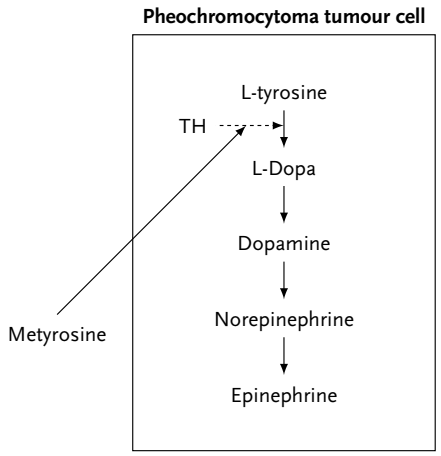
METYROSINE

Metyrosine (α-methylparatyrosine) is a competitive inhibitor of tyrosine hydroxylase, the key enzyme in catecholamine synthesis, catalysing the conversion of l-tyrosine into L-Dopa (*figure 2*).[42] It is usually chosen as a second-line drug, if blood pressure cannot be adequately controlled with α- and β-antagonists. The regular dose is 0.5 to 4 g per day. Side effects of metyrosine are fatigue, diarrhoea, anxiety, depressive mood and crystalluria. Two retrospective studies suggested that addition of metyrosine to phenoxybenzamine results in a better intraoperative blood pressure control, less need for intraoperative phentolamine and a decreased postoperative demand of vasopressors.[23,43]

PREOPERATIVE VOLUME THERAPY

Based on the concept that phaeochromocytoma is accompanied with a reduced intravascular volume, it has been common practice to increase sodium intake (orally or intravenously) simultaneously with antihypertensive therapy. However, study data supporting this clinical approach are limited. Retrospective studies demonstrated fewer complications and less use of vasopressor agents during the postoperative period in patients who received preoperative volume therapy compared with those who did not.[44,45] It should be noted that these studies included historic controls and, therefore, observed benefits could also be the result of other factors, such as improvements in surgical and anaesthetic techniques during the study period.

Figure 2. *Metyrosine inhibits the synthesis of norepinephrine and epinephrine by inhibiting the enzyme tyrosine hydroxylase (TH) which catalyses the conversion of L-tyrosine into L-Dopa*



PREOPERATIVE MANAGEMENT OF PHAEOCHROMOCYTOMA IN THE NETHERLANDS

The different preoperative treatment protocols for patients with a phaeochromocytoma which are currently applied by the eight Dutch university medical centres are described in *table 2*. It is shown that the choice between phenoxybenzamine and doxazosin is equally distributed, whereas calcium channel blockers are not prescribed. Intravenous volume therapy to prevent postoperative hypotension is part of most protocols. Preoperative preparation takes at least seven days in the majority of university medical centres, which also depends on the waiting time before surgery. Patients were routinely admitted to hospital in some centres, but most often preoperative treatment was started in the outpatient clinic.

Table 2. *Preoperative management of phaeochromocytoma in the university medical centres in the Netherlands*

Centre	α-antagonist	β-antagonist	OpC/HS	IV volume
Rotterdam, Erasmus MC	Doxazosin	Always	OpC/HS	On indication
Nijmegen, UMCN St Radboud	Phenoxybenzamine	Always	HS	Always
Maastricht, UM	Phenoxybenzamine	Tachycardia	HS	On indication
Amsterdam, AMC	Phenoxybenzamine	Always	OpC	Always
Utrecht, UMCU	Doxazosin	Always	OpC	Always
Leiden, LUMC	Doxazosin	Tachycardia	OpC/HS	Always
Amsterdam, VUMC	Doxazosin	Tachycardia	OpC	Always
Groningen, UMCG	Doxazosin	Tachycardia	OpC/HS	Always

OpC = outpatient clinic, HS = hospital stay, IV = intravenous.

CONCLUSION

During the past decades resection of a phaeochromocytoma has been accompanied with a major decrease in the perioperative morbidity and mortality. Although randomised, prospective, controlled trials are lacking, data from most retrospective studies demonstrate that preoperative administration of effective antihypertensive agents has contributed to this improvement in surgical outcome. In the absence of solid study data, the choice for a particular blood pressure lowering regimen is also based on pathophysiological considerations and personal experience. Both the nonselective α-antagonist phenoxybenzamine and the selective α_1-antagonist doxazosin are very effective agents for preoperative blood pressure regulation in patients with phaeochromocytoma. If tachycardia occurs, addition of a β-antagonist is often indicated.

ACKNOWLEDGEMENTS

We are indebted to the following colleagues for providing information on preoperative treatment protocols: T.W. van Haeften, MD, PhD, University Medical Centre Utrecht; M.S.P. Huijberts, MD, PhD, University Hospital Maastricht; J.W. Lenders, MD, PhD, Radboud University Nijmegen Medical Centre; P. Lips, MD, PhD, VU University Medical Centre Amsterdam; A.H. van den Meiracker, MD, PhD, Erasmus University Medical Centre Rotterdam; J.A. Romijn, MD, PhD, Leiden University Medical Centre; W.J. Wiersinga, MD, PhD, Academic Medical Centre, University of Amsterdam.

REFERENCES

1. Lenders JWM, Eisenhofer G, Manelli M, Pacak K. Phaeochromocytoma. Lancet 2005;366:665-75.
2. Bravo EL, Tagle R. Phaeochromocytoma: state-of-the-art and future prospects. Endocr Rev 2003;24:539-53.
3. Prys-Roberts C. Phaeochromocytoma--recent progress in its management. Br J Anaesth 2000;85:44-57.
4. Lips CJ, Struyvenberg A, van Rijn HJ, van Rijk PP, Ionescu TI, van Vroonhoven TJ. [Phaeochromocytoma; current viewpoints on diagnosis and therapy]. Ned Tijdschr Geneeskd 1991;135:166-71.
5. Bravo EL. Evolving concepts in the pathophysiology, diagnosis, and treatment of phaeochromocytoma. Endocr Rev 1994;15:356-68.
6. Gupta KK. Letter: Phaeochromocytoma and myocardial infarction. Lancet 1975;1:281-2.
7. Hull CJ. Phaeochromocytoma. Diagnosis, preoperative preparation and anaesthetic management. Br J Anaesth 1986;58:1453-68.
8. Radtke WE, Kazmier FJ, Rutherford BD, Sheps SG. Cardiovascular complications of phaeochromocytoma crisis. Am J Cardiol 1975;35:701-5.
9. Saegesser F. [Cesar Roux (1857-1934) and his times]. Rev Med Suisse Romande 1984;104:403-64.
10. Apgar V, Papper EM. Phaeochromocytoma. Anesthetic management during surgical treatment. AMA Arch Surg 1951;62:634-48.
11. Kinney MA, Narr BJ, Warner MA. Perioperative management of phaeochromocytoma. J Cardiothorac Vasc Anesth 2002;16:359-69.
12. Fernandez-Cruz L, Taura P, Saenz A, Benarroch G, Sabater L. Laparoscopic approach to phaeochromocytoma: hemodynamic changes and catecholamine secretion. World J Surg 1996;20:762-8.
13. Newell KA, Prinz RA, Brooks MH, Glisson SN, Barbato AL, Freeark RJ. Plasma catecholamine changes during excision of phaeochromocytoma. Surgery 1988;104:1064-73.
14. Tauzin-Fin P, Sesay M, Gosse P, Ballanger P. Effects of perioperative alpha1 block on haemodynamic control during laparoscopic surgery for phaeochromocytoma. Br J Anaesth 2004;92:512-7.
15. Kinney MA, Warner ME, van Heerden JA, et al. Perianesthetic risks and outcomes of phaeochromocytoma and paraganglioma resection. Anesth Analg 2000;91:1118-23.
16. Bos JC, Toorians AW, van Mourik JC, van Schijndel RJ. Emergency resection of an extra-adrenal phaeochromocytoma: wrong or right? A case report and a review of literature. Neth J Med 2003;61:258-65.
17. Ulchaker JC, Goldfarb DA, Bravo EL, Novick AC. Successful outcomes in phaeochromocytoma surgery in the modern era. J Urol 1999;161:764-7.
18. Boutros AR, Bravo EL, Zanettin G, Straffon RA. Perioperative management of 63 patients with phaeochromocytoma. Cleve Clin J Med 1990;57:613-7.
19. Iijima T, Takagi T, Iwao Y. An increased circulating blood volume does not prevent hypotension after phaeochromocytoma resection. Can J Anaesth 2004;51:212-5.
20. Stenstrom G, Kutti J. The blood volume in phaeochromocytoma patients before and during treatment with phenoxybenzamine. Acta Med Scand 1985;218:381-7.
21. Orchard T, Grant CS, van Heerden JA, Weaver A. Phaeochromocytoma – continuing evolution of surgical therapy. Surgery 1993;114:1153-8.

22. Remine WH, Chong GC, van Heerden JA, Sheps SG, Harrison EG Jr. Current management of phaeochromocytoma. Ann Surg 1974;179:740-8.

23. Steinsapir J, Carr AA, Prisant LM, Bransome ED Jr. Metyrosine and phaeochromocytoma. Arch Intern Med 1997;157:901-6.

24. Loh KC, Shlossberg AH, Abbott EC, Salisbury SR, Tan MH. Phaeochromocytoma: a ten-year survey. QJM 1997;90:51-60.

25. Stenstrom G, Haljamae H, Tisell LE. Influence of pre-operative treatment with phenoxybenzamine on the incidence of adverse cardiovascular reactions during anaesthesia and surgery for phaeochromocytoma. Acta Anaesthesiol Scand 1985; 29:797-803.

26. Russell WJ, Metcalfe IR, Tonkin AL, Frewin DB. The preoperative management of phaeochromocytoma. Anaesth Intensive Care 1998;26:196-200.

27. Luo A, Guo X, Yi J, Ren H, Huang Y, Ye T. Clinical features of phaeochromocytoma and perioperative anesthetic management. Chin Med J (Engl) 2003;116:1527-31.

28. Prys-Roberts C, Farndon JR. Efficacy and safety of doxazosin for perioperative management of patients with phaeochromocytoma. World J Surg 2002;26:1037-42.

29. Kocak S, Aydintug S, Canakci N. Alpha blockade in preoperative preparation of patients with phaeochromocytomas. Int Surg 2002;87:191-4.

30. Nicholson JP Jr, Vaughn ED Jr, Pickering TG, et al. Phaeochromocytoma and prazosin. Ann Intern Med 1983;99:477-9.

31. Crago RM, Eckholdt JW, Wismell JG. Phaeochromocytoma. Treatment with alpha- and beta-adrenergic blocking drugs. JAMA 1967;202:870-4.

32. Ross EJ, Prichard BN, Kaufman L, Robertson AI, Harries BJ. Preoperative and operative management of patients with phaeochromocytoma. BMJ 1967;1:191-8.

33. Prichard BN, Ross EJ. Use of propranolol in conjunction with alpha receptor blocking drugs in phaeochromocytoma. Am J Cardiol 1966;18:394-8.

34. Briggs RS, Birtwell AJ, Pohl JE. Hypertensive response to labetalol in phaeochromocytoma. Lancet 1978;1:1045-6.

35. Shupak RC. Difficult anesthetic management during phaeochromocytoma surgery. J Clin Anesth 1999;11:247-50.

36. Perry LB, Gould AB Jr. The anesthetic management of phaeochromocytoma effect of preoperative adrenergic blocking drugs. Anesth Analg 1972;51:36-40.

37. Proye C, Thevenin D, Cecat P, et al. Exclusive use of calcium channel blockers in preoperative and intraoperative control of phaeochromocytomas: hemodynamics and free catecholamine assays in ten consecutive patients. Surgery 1989;106:1149-54.

38. Favre L, Forster A, Fathi M, Vallotton MB. Calcium-channel inhibition in phaeochromocytoma. Acta Endocrinol (Copenh) 1986;113:385-90.

39. Lenders JW, Sluiter HE, Thien T, Willemsen J. Treatment of a phaeochromocytoma of the urinary bladder with nifedipine. Br Med J (Clin Res Ed) 1985;290:1624-5.

40. Pinto JE, Trifaro JM. The different effects of D-600 (methoxyverapamil) on the release of adrenal catecholamines induced by acetylcholine, high potassium or sodium deprivation. Br J Pharmacol 1976;57:127-32.

41. Lebuffe G, Dosseh ED, Tek G, et al. The effect of calcium channel blockers on outcome following the surgical treatment of phaeochromocytomas and paragangliomas. Anaesthesia 2005;60:439-44.

42. Engelman K, Jequier E, Udenfriend S, Sjoerdsma A. Metabolism of alpha-methyltyrosine in man: relationship to its potency as an inhibitor of catecholamine biosynthesis. J Clin Invest 1968;47:568-76.

43. Perry RR, Keiser HR, Norton JA, et al. Surgical management of phaeochromocytoma with the use of metyrosine. Ann Surg 1990;212:621-8.

44. Deoreo GA Jr, Stewart BH, Tarazi RC, Gifford RW Jr. Preoperative blood transfusion in the safe surgical management of phaeochromocytoma: a review of 46 cases. J Urol 1974;111:715-21.

45. Desmonts JM, le Houelleur J, Remond P, Duvaldestin P. Anaesthetic management of patients with phaeochromocytoma. A review of 102 cases. Br J Anaesth 1977;49:991-8.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Long-term clinical outcome of patients with diabetes proposed for coronary revascularisation

J.R. Timmer[1], A. Breeman[1], J.P. Ottervanger[1*], E.P. de Kluiver[1], P.W. Boonstra[2], F. Zijlstra[3]

[1]Department of Cardiology, Isala Klinieken, Zwolle, the Netherlands, Departments of [2]Thoracic Surgery and [3]Cardiology, Thoraxcentre, University Medical Centre Groningen, the Netherlands,
[*]corresponding author: tel.: +31 (0)38-424 21 98, fax: +31 (0)38-424 32 22, e-mail: v.r.c.derks@isala.nl

ABSTRACT

Background: The optimal method of revascularisation in diabetic patients with coronary artery disease (CAD) remains controversial. It was our aim to evaluate long-term outcome in diabetic patients with CAD in daily practice, in whom an invasive approach was considered.
Methods: A prospective follow-up study of patients with CAD in whom a coronary revascularisation procedure was considered. Follow-up data were obtained on the vital status up to ten years after inclusion.
Results: Of the 872 included patients, a total of 107 patients (12%) had diabetes. Patients with diabetes were older and more frequently female. Long-term mortality was higher in diabetics than nondiabetics (36 *vs* 25%, p=0.01). This association was observed in both medically treated patients (65 *vs* 31%, p=0.01) and in those treated by percutaneous coronary intervention (41 *vs* 24%, p=0.02). There was, however, no difference in mortality in diabetes *vs* nondiabetes patients after coronary artery bypass grafting (24 *vs* 24%, p=0.89). Multivariate analysis did not change these findings.
Conclusion: Diabetic patients with significant CAD had a higher long-term mortality compared with patients without diabetes. In patients with diabetes, survival was highest after coronary artery bypass grafting and appeared to be comparable between diabetic and nondiabetic patients. Complete revascularisation may decrease the influence of diabetes on survival.

KEYWORDS

Diabetes, CABG, revascularisation

INTRODUCTION

Diabetes is associated with impaired outcome after coronary revascularisation.[1,2] The optimal method of revascularisation remains controversial. In the BARI trial, coronary artery bypass grafting (CABG), when compared with percutaneous coronary intervention (PCI), was associated with a significant seven-year mortality reduction in patients with diabetes.[3] However, this long-term benefit was not confirmed by other randomised trials.[4,5] Furthermore, patients in daily practice may differ from those included in randomised trials, as was shown in the registry of the BARI trial.[6] To evaluate the long-term outcome of diabetic patients with coronary artery disease (CAD) in daily practice in whom an invasive approach was considered, we carried out a follow-up study of patients included in the DUCAT (Dutch Inventory of Invasive Coronary Atherosclerosis Treatments) study.

PATIENTS AND METHODS

The DUCAT study was initiated in 1992 with the purpose to determine how appropriate treatment decisions are concerning invasive treatment of patients with CAD.[7] Assessment of appropriateness of medical decisions was achieved using the RAND/UCLA method.[8] Six cardiothoracic surgeons and six interventional cardiologists from 12 heart centres in the Netherlands were asked to participate in a panel to determine appropriateness of treatment decisions in all consecutive patients presented to ten heart teams, consisting of at least one surgeon and one interventional cardiologist. This panel method has been proven to be consistent and reliable in assessing appropriateness.[8]

Enrolment began in February 1992 for a period of three months. Each case was presented by a clinical cardiologist in person or by letter, fax, or telephone, and eventually led to an intention-to-treat decision in favour of PCI, CABG, or medical treatment. All presentations were based on clinical data and coronary angiographic results. Enrolment was approved for all patients with significant CAD, defined by the DUCAT panel as a minimum of 50% narrowing of the left main coronary artery, or at least one artery with 70% narrowing and other arteries with 50% narrowing in multivessel disease, and one artery with 70% narrowing in one-vessel disease. Patients who had previously had CABG or in whom CABG was to be combined with other surgery (cardiac or general) were excluded. During presentation to the heart team, several variables were collected, including demographics, medical history, risk factors for CAD, symptomatology, ischaemia detection tests, coronary angiographic evaluation, left ventricular function tests, urgency status and intention of treatment. The main findings of the DUCAT study have been published previously.[7,8] In summary, 3646 consecutive patients were included. Unstable angina was the most appropriate clinical status for intervention, whereas asymptomatic coronary disease was the least.

The present study is a two-centre follow-up study of all patients consecutively presented to the heart team of the heart centres of Zwolle and Groningen in the Netherlands. In these two centres, a total of 1047 patients were presented to the heart teams during the study period. Follow-up data were obtained on the vital status up to ten years after inclusion. Follow-up data were collected via the registry office, the general practitioner or via a direct contact with the patient or his relatives by telephone.

DEFINITIONS

Diabetes was defined as the use of oral hypoglycaemic agents, insulin, or a diabetes-related diet with diabetes documented in the medical history. The presence of hypertension and a positive family history were derived from the data in the medical history. Lipid disorder was present when lipid-lowering medication was used or when stated in the medical history. Peripheral vascular disease was defined as the presence of symptomatic claudication or a history of peripheral vascular surgery. Stroke was defined as either an ischaemic or a haemorrhagic cerebral vascular accident with permanent sequelae. Decreased LV function was defined as an ejection fraction ≤40%. Type C lesion was defined as a stenosis longer than 20 mm, or with rugged contours or tortuous shape, or located at spots not readily accessible for a catheter, or total vessel occlusions older than three months.

STATISTICS

Differences between group means were tested by two-tailed Student's t test. A χ^2 statistic was calculated to test differences between proportions. Survival functions were calculated, using the Kaplan-Meier product limit method. Mantel-Cox (or log-rank) test was applied to evaluate the differences between survival functions.

Multivariate Cox proportional-hazards regression analysis was applied to assess the independent relation between revascularisation strategy and ten-year survival after adjustment for baseline characteristics.

RESULTS

Patients

Of the 1057 included patients, ten cases were censored because they were presented to the heart team for the second time. Of the remaining 1047 patients, 877 (84%) met the inclusion criteria and were enrolled in the study. The diabetic status was unknown for five patients; these patients were not included in our analysis. Our analysis therefore consisted of 872 patients. A total of 107 patients (12%) had diabetes. Patients with diabetes were older (66 ± 8 *vs* 62 ± 10 year, p<0.001), were more frequently female (55 *vs* 21%, p<0.001) and had a higher prevalence of hypertension (46 *vs* 27%, p<0.001) when compared with patients without diabetes. Furthermore, patients with diabetes more often had multivessel CAD. Differences in baseline characteristics between patients with and without diabetes are shown in *table 1*.

Coronary revascularisation

In patients without diabetes, PCI was recommended in 333 patients (44%), CABG in 333 patients (44%) and a conservative approach was advised in 99 patients (13%). In patients with diabetes the type of revascularisation was comparable with those without diabetes, with percentages of patients recommended for PCI, CABG or conservative approach of 36, 48 and 16%, respectively. There were several differences between diabetic patients treated conservatively and those treated invasively. There were no differences in baseline characteristics between diabetic patients treated with PCI and those treated with CABG with regard to age, sex, risk factors, coronary history, comorbidity or left ventricular ejection fraction (LVEF). However, patients with diabetes treated with PCI more often had single-vessel disease (56 *vs* 4%, p<0.001) compared with those treated with CABG.

Long-term mortality

In the total study group, long-term mortality was associated with increasing age, no revascularisation, diabetes (36 *vs* 25%, p=0.01), peripheral artery disease

Table 1. *Baseline characteristics of all patients according to diabetic status*

	Diabetes (n=107)	Nondiabetes (n=765)	P value
Age in years (mean SD)	66 ± 8	62 ± 10	<0.001
Male	48 (44.9%)	601 (78.6%)	<0.001
Risk factors			
• Hypertension	49 (45.8%)	208 (27.3%)	<0.001
• Smoking	17 (18.9%)	209 (29.7%)	0.033
• Lipid disorder	35 (35.7%)	261 (37.1%)	0.786
• Family history	24 (28.9%)	278 (42.4%)	0.018
• Obesity	17 (21.3%)	77 (13.2%)	0.054
Coronary history			
• Previous myocardial infarction	35 (32.7%)	292 (38.3%)	0.266
• Previous PCI	13 (12.1%)	83 (10.9%)	0.694
Comorbidity			
• COPD	12 (11.2%)	66 (8.7%)	0.389
• Peripheral vascular disease	17 (15.9%)	70 (9.2%)	0.030
• Stroke	12 (11.2%)	41 (5.4%)	0.018
Vessels diseased			
• One vessel	28 (26.2%)	275 (35.9%)	0.047
• Two vessels	36 (33.6%)	214 (28.0%)	0.224
• Three vessels	37 (34.6%)	223 (29.2%)	0.250
• Left main coronary artery	6 (5.6%)	53 (6.9%)	0.610
• Type C lesion	58 (54.2%)	382 (49.9%)	0.408
LVEF 20 to 40%	17 (16.3%)	116 (15.6%)	0.843
<20%	5 (4.8%)	20 (2.7%)	0.218

SD = standard deviation; PCI = percutaneous coronary intervention; COPD = chronic obstructive pulmonary disease.

and an LVEF ≤40%. As compared with revascularisation by either PCI or CABG, medically treated patients had an increased mortality. The increased mortality in patients with diabetes was observed in both medically treated patients (n=116) (diabetes 65 *vs* nondiabetes 31%, p=0.01) and in those who received PCI (n=372) (diabetes 41 *vs* nondiabetes 24%, p=0.02). There was, however, no difference in mortality between diabetes and nondiabetes after CABG (n=384) (24 *vs* 24%, p=0.89). Survival curves of patients with and without diabetes according to type of revascularisation are shown in *figures 1* to *3*.

Figure 2. *Ten years' follow-up of 372 PCI-treated patients*

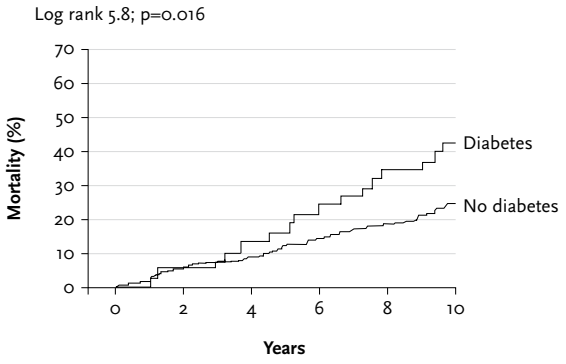


Figure 1. *Ten years' follow-up of 116 medically treated patients*

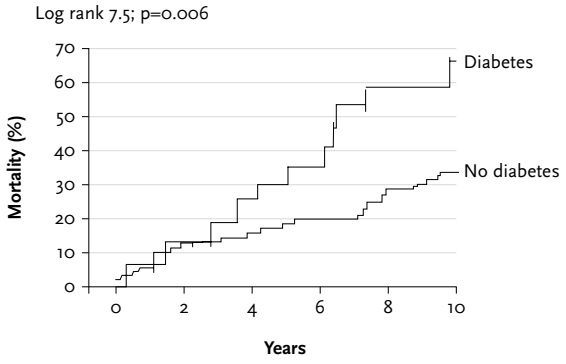
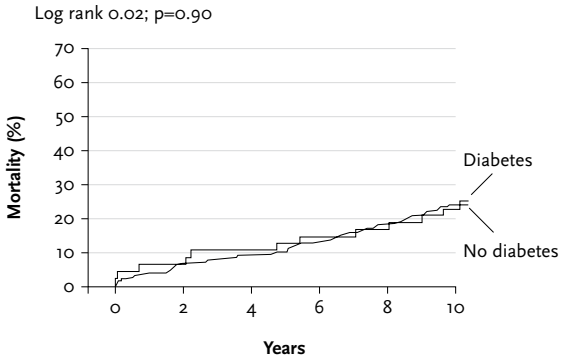


Figure 3. *Ten years' follow-up of 384 CABG-treated patients*



Multivariate analysis

To study the independent prognostic importance of diabetes on clinical outcome, multivariate analysis was performed. We included age, gender and all univariate predictors of long-term mortality in the multivariate model. After multivariate analysis increasing age, diabetes and a decreased LV function were independent predictors of long-term mortality (*table 2*). The independent predictive value of diabetes was most pronounced in patients treated without revascularisation and in those treated with PCI (OR 2.1, 95% CI 1.3 to 3.3). In patients treated with CABG, diabetes was not an independent predictor of long-term mortality (OR 1.2, 95% CI 0.6 to 2.4).

Table 2. *Independent predictors of mortality*

	OR	95% CI
Male gender	1.36	0.98 to 1.89
Age (per year)	1.09	1.07 to 1.12
Conservative treatment[#]	1.24	0.86 to 1.79
Diabetes	1.46	1.01 to 2.13
Peripheral vascular disease	1.28	0.85 to 1.91
Decreased left ventricle function	1.58	1.15 to 2.18

[#]As compared with revascularisation by either PCI or CABG. Adjusted for differences in the other variables. OR = odds ratio; CI = confidence limit.

DISCUSSION

In patients with coronary artery disease in whom an invasive approach was considered, diabetes was associated with increased long-term mortality. However, in patients treated with CABG this association was not observed. Our analysis reflects real-world clinical practice and has additional value regarding the still ongoing debate about the optimal method of revascularisation for patients with diabetes.

Diabetes *vs* nondiabetes

Patients with diabetes had a higher long-term mortality compared with patients without diabetes. This could be due to differences in baseline characteristics. In general, patients with diabetes were older, were more often female and had a higher prevalence of hypertension. However, after multivariate analysis diabetes was still a significant predictor of mortality. There may be several mechanisms for this increase in mortality. A procoaguable state and more unfavourable lipid levels in diabetic patients might play a role.[9,10] Atherosclerotic coronary abnormalities may be more progressive in diabetes. Furthermore, pre-existing left ventricular failure, either diastolic or systolic, may contribute to an increase in mortality.[11] Moreover, glycometabolic disturbances during acute coronary events increase infarct size and might predispose to ventricular arrhythmias.[12] Interestingly, in patients with extensive CAD, who are generally treated with CABG, the difference in mortality between diabetes and nondiabetes seems to be much less clear.[13]

PCI as revascularisation strategy

Mortality was higher in PCI-treated patients with diabetes compared with those without diabetes. There may be several explanations for this difference. Patients with diabetes have smaller calibre vessels and higher rates of restenosis than patients without diabetes.[14] Furthermore, more progressive atherosclerosis in diabetes may also affect coronary segments not significantly stenosed at the time of the initial decision to perform revascularisation. As diabetic patients already have a comprised LV function, display impaired preconditioning and have glycometabolic disturbances potentially increasing ischaemic myocardial damage, they might be more prone to die when suffering subsequent coronary events.[11,15]

CABG as revascularisation strategy

There was no difference in mortality in diabetic patients treated with CABG compared with nondiabetic patients treated with CABG. Previous studies show conflicting results. Several studies report that diabetes is associated with a worse outcome after CABG.[1,16] The randomised EAST trial and the recently published study by Calafiore *et al.*, however, did not find an association between diabetes and a long-term adverse prognosis after CABG.[5,17]

Revascularisation in diabetes

In patients with diabetes, those treated medically had the highest mortality, whereas the lowest mortality was found in diabetic patients treated with CABG. Differences in baseline characteristics between medically treated patients and those undergoing revascularisation may partly explain the differences in outcome. Medically treated patients were older and had a higher prevalence of peripheral artery disease and COPD than those treated with coronary revascularisation. The improved prognosis after CABG in diabetic patients can be caused by the fact that PCI in diabetes is associated with a higher restenosis rate, whereas graft patency after four years between patients with and without diabetes may be comparable.[18] Also, incomplete revascularisation might be detrimental in diabetic patients treated with PCI. Coronary artery bypass grafting may be superior in reaching complete revascularisation leaving PCI-treated patients with an increased area at risk for future ischaemic events.[19] Furthermore, there seems to be a protective effect from the use of an internal thoracic artery (ITA) in CABG, especially in patients with diabetes.[20] The mortality risk after myocardial infarction in diabetic patients without

ITA is much higher when compared with diabetic patients who had received revascularisation with an ITA. In our study the majority of patients were treated with an ITA.
It is still unclear whether the type of antidiabetic treatment used interacts with the success of different revascularisation procedures. Although the use of drug-eluting stents has reduced the occurrence of restenosis in diabetic patients, diabetic patients using insulin still appear to be at an increased risk.[21,22] It is possible that particularly this patient group could benefit from surgical revascularisation compared with PCI.[23]

Other studies

In the BARI trial, 1829 patients with multivessel disease were randomised to CABG or PCI.[3] This study found a better seven-year survival in diabetic patients (n=353) treated with CABG compared with PCI (76 *vs* 56%). However, other randomised trials, as the CABRI trial and the EAST trial, did not find a significant beneficial effect after CABG compared with PCI in diabetic patients.[4,5] Moreover, the RITA trial including 62 patients with diabetes found a nonsignificantly worse outcome for diabetic patients randomised to CABG compared with PCI.[24] Observational studies also found contradicting evidence regarding the optimal method of reperfusion. The Duke and EMORY analyses did not find a benefit for CABG *vs* PCI in diabetic patients, although insulin-treated diabetes seemed to benefit from CABG.[1,2] The MAHI study found an unadjusted survival benefit of diabetic patients when treated with CABG compared with PCI.[25] Interestingly, in diabetic patients from the registry of the BARI trial there was no significant difference in mortality between the two revascularisation methods.[6] Niles *et al.*, however, did find a significant reduction in mortality in more than 7000 diabetic patients treated with CABG *vs* PCI after five years of follow-up.[26] Our study also found a survival benefit for diabetic patients when treated with CABG compared with PCI after a long follow-up period of ten years.

STUDY LIMITATIONS

This was an observational study without randomisation. This could have led to differences in unmeasured baseline characteristics for which no correction or adjustment could be made.
Unfortunately, no detailed information about medication use or the type of antidiabetic treatment was available. During the study period, intracoronary stenting and treatment with glycoprotein IIb-IIIa inhibitors or clopidogrel were not available. These new therapeutic modalities may well have a profound effect on clinical outcome and may, in particular, improve clinical outcome in patients with diabetes when treated with PCI.[27,28]

CONCLUSION

Diabetic patients with significant CAD had a higher long-term mortality compared with patients without diabetes. In patients with diabetes survival was highest after coronary artery bypass grafting and appeared to be comparable between diabetic and nondiabetic patients. Complete revascularisation may decrease the influence of diabetes on survival.

REFERENCES

1. Barsness GW, Peterson ED, Ohman EM, et al. Relationship between diabetes and long-term survival after coronary bypass and angioplasty. Circulation 1997;96:2551-6.
2. Weintraub WS, Stein B, Kosinski A, et al. Outcome of coronary bypass surgery vs coronary angioplasty in diabetic patients with multivessel coronary artery disease. J Am Coll Cardiol 1998;31:10-9.
3. The BARI Investigators. Seven-year outcome in the Bypass Angioplasty Revascularization Investigation (BARI) by treatment and diabetic status. J Am Coll Cardiol 2000;35:1122-9.
4. Kurbaan AS, Bowker TJ, Ilsley CD, Sigwart U, Rickards AF, on behalf of the CABRI Investigators. Difference in the mortality of the CABRI diabetic and nondiabetic populations and its relation to coronary artery disease and the revascularization mode. (Coronary Angioplasty vs Bypass Revascularization Investigation). Am J Cardiol 2001;87:947-50.
5. King SB III, Kosinski AS, Guyton RA, Lembo NJ, Weintraub WS. Eight-year mortality in the Emory Angioplasty vs Surgery Trial (EAST). J Am Coll Cardiol 2000;35:1116-21.
6. Detre KM, Guo P, Holubkov R, et al. Coronary revascularization in diabetic patients: a comparison of the randomized and observational components of the Bypass Angioplasty Revascularization Investigation (BARI). Circulation 1999;99:633-40.
7. Rigter H, Meijler AP, McDonnell J, Scholma JK, Bernstein SJ. Indications for coronary revascularisation: a Dutch perspective. Heart 1997;77:211-8.
8. Meijler AP, Rigter H, Bernstein SJ, et al. The appropriateness of intention to treat decisions for invasive therapy in coronary artery disease in The Netherlands. Heart 1997;77:219-24.
9. Winocour PD. Platelet abnormalities in diabetes. Diabetes 1992;41(suppl 2):26-31.
10. Kreisberg RA. Diabetic dyslipidemia. Am J Cardiol 1998;82:U67-73.
11. Norhammar A, Malmberg K. Heart failure and glucose abnormalities: an increasing combination with poor functional capacity and outcome. Eur Heart J 2000;21:1293-4.
12. Oliver MF, Opie LH. Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. Lancet 1994;343:155-8.
13. Ishihara M, Sato H, Kawagoe T, et al. Impact of diabetes mellitus on long term survival after acute myocardial infarction in patients with single vessel disease. Heart 2001;86:133-8.
14. Van Belle E, Bauters C, Hubert E, et al. Restenosis rates in diabetic patients: a comparison of coronary stenting and balloon angioplasty in native coronary vessels. Circulation 1997;96:1454-60.
15. Ishihara M, Inoue I, Kawagoe T, et al. Diabetes prevents ischemic preconditioning in patients with a first acute anterior wall myocardial infarction. J Am Coll Cardiol 2001;38:1007-11.
16. Brooks MM, Jones RH, Bach RG, et al. Predictors of mortality and mortality from cardiac causes in the bypass angioplasty revascularization investigation (BARI) randomized trial and registry. For the BARI Investigators. Circulation 2000;101:2682-9.
17. Calafiore AM, Di Mauro M, Di Giammarco G, et al. Effect of diabetes on early and late survival after isolated first coronary bypass surgery in multivessel disease. J Thorac Cardiovasc Surg 2003;125:144-54.

18. Schwartz L, Kip KE, Frye RL, Alderman EL, Schaff HV, Detre KM. Coronary bypass graft patency in patients with diabetes in the Bypass Angioplasty Revascularization Investigation (BARI). Circulation 2002;106:2652-8.

19. Zhao XQ, Brown BG, Stewart DK, et al. Effectiveness of revascularization in the Emory angioplasty vs surgery trial. A randomized comparison of coronary angioplasty with bypass surgery. Circulation 1996;93:1954-62.

20. Detre KM, Lombardero MS, Brooks MM, et al. The effect of previous coronary-artery bypass surgery on the prognosis of patients with diabetes who have acute myocardial infarction. Bypass Angioplasty Revascularization Investigation Investigators. N Engl J Med 2000;342:989-97.

21. Ortolani P, Ardissino D, Cavallini C, et al. Effect of sirolimus-eluting stent in diabetic patients with small coronary arteries. Am J Cardiol 2005;96:1393-8.

22. Yang TH, Park SW, Hong MK, et al. Impact of diabetes mellitus on angiographic and clinical outcomes in the drug-eluting stents era. Am J Cardiol 2005;96:1389-92.

23. Brandt M, Harder K, Walluscheck KP, et al. Coronary artery bypass surgery in diabetic patients. J Card Surg 2004;19:36-40.

24. Henderson RA, Pocock SJ, Sharp SJ, et al. Long-term results of RITA-1 trial: clinical and cost comparisons of coronary angioplasty and coronary-artery bypass grafting. Randomised Intervention Treatment of Angina. Lancet 1998;352:1419-25.

25. Gum PA, O'Keefe JH Jr, Borkon AM, et al. Bypass surgery vs coronary angioplasty for revascularization of treated diabetic patients. Circulation 1997;96(suppl II):7-10.

26. Niles NW, McGrath PD, Malenka D, et al. Survival of patients with diabetes and multivessel coronary artery disease after surgical or percutaneous coronary revascularization: results of a large regional prospective study. Northern New England Cardiovascular Disease Study Group. J Am Coll Cardiol 2001;37:1008-15.

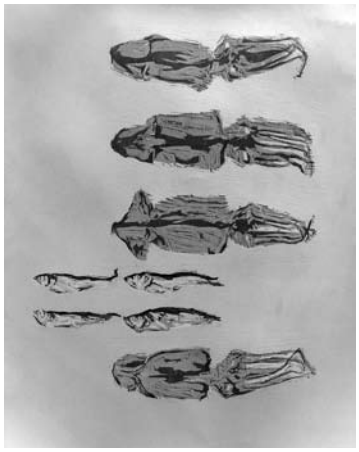
27. Van Belle E, Perie M, Braune D, et al. Effects of coronary stenting on vessel patency and long-term clinical outcome after percutaneous coronary revascularization in diabetic patients. J Am Coll Cardiol 2002;40:410-7.

28. Lincoff AM, Tcheng JE, Califf RM, et al. Sustained suppression of ischemic complications of coronary intervention by platelet GP IIb/IIIa blockade with abciximab: one-year outcome in the EPILOG trial. Evaluation in PCI to Improve Long-term Outcome with abciximab GP IIb/IIIa blockade. Circulation 1999;99:1951-8.

ABOUT THE COVER

'Gedroogde inktvis'

Manuela Jalimsing



Manuela Jalimsing was born in 1971 in Paramaribo. She now lives and works in The Hague. Manuele attended the College of Art in Utrecht and the Royal Academy of Art in The Hague.

Her work can be seen in many group and solo exhibitions in the Netherlands.

With the shape of fish, she designs a composition on a surface. The expression of the fish and the transparency due to the drying in salt attract her. Every fish is put in its own position, thus creating a certain tension, trying to reach a balance. After observation the images are put onto paper. She often uses desiccated fish, bought at the Chinese supermarket.

Her passion for the wooden print is probably based on the fact that it is a near to nature and practical technique in the course of which you can create images without using lots of material.

In her childhood, fish was one of her main sources of inspiration. Her father was a fisherman on the Atlantic Ocean from the age of eleven, just as his father and his brothers. The many stories about the rough sea and his catches made a great of impression on her.

During her studies at the Academy of Art, Jalimsing was pregnant and she avoided the studios because of the chemical substances. When making wooden prints she only used a board, a wooden spoon and a gouge, and she experienced the natural art of wooden print.

Later, she attended a workshop by a Japanese master, who taught her how to use only pure materials.

An original print (71 x 100 cm) is available at a price of € 525 and can be ordered from Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands, e-mail: Galerie-Unita @planet.nl, www.galerie-unita.com.

Netherlands
The Journal of Medicine

CASE REPORT

Fever and high lactate dehydrogenase in HIV-positive patients from the Antilles and Surinam: histoplasmosis?

E.J.G. Peters[1*], R.H. Kauffmann[1], P. Blok[2]

Departments of [1]Internal Medicine and [2]Clinical Pathology, Haga Hospital, location Leyenburg, the Hague, the Netherlands, [*]corresponding author: tel.: +31 (0)70-359 20 00, e-mail: e.j.g.peters@lumc.nl

ABSTRACT

We describe four cases of HIV-positive patients, two from Surinam, one from the Dutch Antilles and one from Nigeria, who presented with a febrile illness and a high lactate dehydrogenase plasma level. In all four, the diagnosis of disseminated histoplasmosis was made, in three of them by liver biopsy. Two patients had retinal abnormalities compatible with a systemic fungal infection. Three patients were treated successfully with antifungal agents. One patient died. Between 2000 and 2006, only 14 patients with HIV have been found to have histoplasmosis in the Netherlands. Although histoplasmosis is not endemic in the Netherlands, physicians are more likely to see cases because of a growing number of HIV-positive immigrants from endemic regions.

KEYWORDS

Histoplasma, HIV, lactate dehydrogenase

INTRODUCTION

Although disseminated histoplasmosis is a common AIDS-defining condition in endemic countries, it is rarely encountered in Northwest Europe.[1] In the Netherlands, histoplasmosis was diagnosed in 14 HIV-positive patients from January 2000 to January 2006 (data from the HIV Monitoring Foundation). Only one patient was born in the Netherlands. The other patients originated from Western Africa (5) and Southern and Central America (8). Since physicians from Western Europe may not be familiar with this life-threatening disease, there may be a fatal delay in diagnosis. In this article, we describe four patients, all of whom had high plasma lactate dehydrogenase (LDH) levels. LDH might be used to decide about the treatment of histoplasmosis empirically before definite identification of the organism is available.

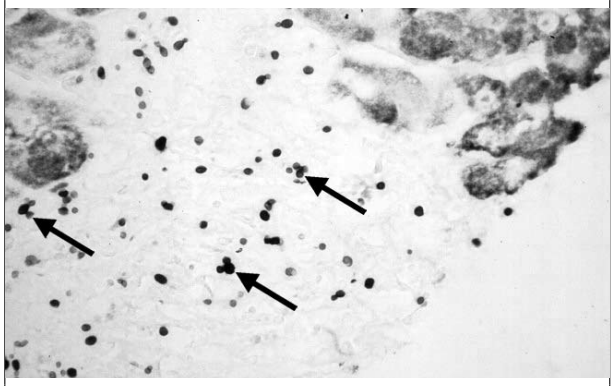
CASE REPORT 1

A 41-year-old Negroid man from Surinam with a two-year history of HIV infection presented with pain in the upper abdomen and fever for a couple of days. Physical examination did not reveal any abnormalities. Laboratory results showed panleukopenia (1.5 x 10^9/l), normocytic anaemia (5.0 mmol/l), thrombopenia (88 x 10^9/l), erythrocyte sedimentation rate (ESR) 135 mm/h, aspartate aminotransferase (ASAT) 50 U/l, alanine aminotransferase (ALAT) 20 U/l, LDH 1413 U/l, and CD4 count 8/mm^3. Microbiological examination commonly used in HIV patients to find the origin of fever remained negative. On bone marrow examination granulomatous lesions were found without any micro-organisms. The patient was first treated with tuberculostatic drugs without effect on the fever. Finally, histoplasmosis was found in a liver biopsy (*figure 1*). He was treated with amphotericin B, followed by itraconazole 200 mg twice daily for four years. The fever resolved within three days. He finally died four years later of other complications of his HIV infection.

CASE REPORT 2

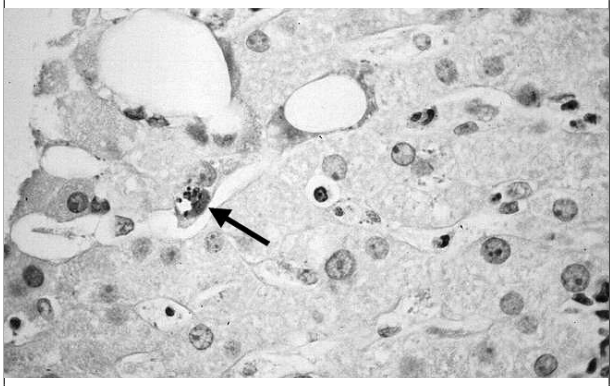
A 54-year-old Negroid woman from Curacao was admitted from another hospital for treatment of progressive renal insufficiency with proteinuria and a coincidentally

Figure 1. *Grocott stain of the liver (630 x) of the patient in the first case report*



***Histoplasma* is abundantly present, some with smaller buds. Examples are highlighted with arrows.**

Figure 2. *PAS coloured liver specimen (630 x) of the patient in the second case report*



The arrow points at the *Histoplasma* lesion.

diagnosed HIV infection. She had a six-year history of diabetes mellitus and had experienced an episode of erythema multiforme of unknown aetiology after a visit to Curacao, four years prior to her admission. She had not returned to Curacao after that last visit. She complained of episodes of fever, fatigue and frontal headache for a few months. She had a systolic ejection murmur. There was no stiffness of the neck. White retinal lesions were seen on fundoscopy. Leucocytes were 5.0 x 10^9/l with lymphopenia (0.6 x 10^9/l), microcytic anaemia (6.4 mmol/l), thrombocytes 288 x 10^9/l, ESR 95 mm/h, creatinine 1100 µmol/l, urea 32 mmol/l, total protein 47 g/l, albumin 14 g/l, ASAT 176 U/l, ALAT 59 U/l, LDH 3715 U/l, γ-glutamyltransferase, 141 U/l, CD4 count 6/mm, HIV-RNA >10^6 copies/ml, and urinary protein 15 g/24h. Standard microbiological and mycobacterial cultures remained negative. Radiological examination with ultrasound and computed tomography (CT) scan showed pleural effusion, enlarged liver, enlarged echogenic kidneys, few iliacal lymph nodes and some ascites. Kidney biopsy revealed HIV-associated nephropathy. Finally, histoplasmosis could be diagnosed from liver biopsy, performed one week after admission (*figure 2*). The patient was treated with itraconazole 200 mg twice daily, but died two days later of multiple organ failure. At autopsy histoplasmosis was found in most of her organs.

CASE REPORT 3

A 26-year-old Negroid Surinam HIV-positive male, who had been in the Netherlands for one year, was admitted because of fever for two weeks, 10 kg weight loss and loss of vision of his left eye. On physical examination an oral *Candida* infection, a skin ulcer on the chin and a palpable liver were found. A white exudate and a retinal detachment were seen at fundoscopy. Leucocytes were 3.6 x 10^9/l with a left shift. Haemoglobin was 6.5 mmol/l, thrombocytes 151 x 10^9/l, creatinine 113 µmol/l, ASAT 242 U/l, ALAT 50 U/l, LDH 3100 U/l, CD4 count 12/mm^3 and HIV-RNA >10^6 copies/ml. Standard examination of blood, faeces and pleural fluid did not yield any micro-organisms. Viral culture of the chin ulcer revealed a herpes simplex type 2 infection. Mild spleen and liver enlargement was seen at ultrasound. The patient refused a liver biopsy. Based on earlier experience, disseminated histoplasmosis was suspected. Amphotericin B was started five days after admission, followed by itraconazole. No antibodies against *Histoplasma* could be detected with an immunodiffusion test. The fever receded and the LDH normalised within two days. Blood cultures grew *Histoplasma capsulatum* 13 days after admission. He was treated with antiviral combination therapy and continued itraconazole. Within three months, CD4 cells rose to 170/mm^3 and HIV-RNA became undetectable. Six months later he was readmitted with extensive cutaneous histoplasmosis after he had stopped all medication.

CASE REPORT 4

A Negroid man from Nigeria had resided in the Netherlands for 3.5 years. He had a history of malaria (*Plasmodium vivax*) and presented with fever and chills after a root canal treatment at the dentist. He reported weight loss of 15 kg in the previous months. On physical examination he had a systolic cardiac murmur at the apex. No abnormalities were found at fundoscopy. A HIV test was positive. Leucocytes were 3.9 with 3.4 granulocytes and 0.5 x 10^9/l mononuclear cells. Haemoglobin was 5.3 mmol/l with a mean cellular volume of 81 fl, thrombocytes 237 x 10^9/l, ESR 128 mm, C-reactive protein 40 U/l, creatinine 92 µmol/l, ASAT 67 U/l, ALAT 28 U/l, LDH 1516 U/l, and

LDH iso-enzymes: lactate dehydrogenase 1 iso-enzyme (LD_1) 270, LD_2 347, LD_3 312, LD_4 174, and LD_5 162 U/l. The CD4 count was 14/mm³ and HIV-RNA >10^6 copies/ml. Initial standard microbiological and mycobacterial cultures remained negative. *Histoplasma* serology was negative. Reticulonodular lesions were seen on plain chest X-ray. CT scan of the neck, thorax and abdomen showed alveolar infiltrates, mediastinal lymphadenopathy and an enlarged spleen (14 cm). Bone marrow aspiration revealed HIV myelopathy and foamy histiocytes with micro-organisms resembling *Histoplasma,* intense iron staining and sideroblasts. Lung, liver and cervical lymph node biopsy all showed *Histoplasma*. Treatment with amphotericin B was started two weeks after admission. One week later, blood cultures grew *Histoplasma*. The fever receded in six days, and he recovered fully. Nine months later, he was in excellent condition on antiviral therapy and itroconazole. His CD4 cells were 370/mm³ and HIV RNA was undetectable.

DISCUSSION

Histoplasmosis encapsulatum is a dimorphic organism. The hyphae or conidia of the organism are inhaled and can reach the alveoli, where they can germinate at 37°C into the yeast phase and invade the body.[2] In tissue, these yeast cells are found in the macrophages as small buds. The acute infection is mostly asymptomatic, but can present as a flu-like illness, with pulmonary symptoms, skin lesions, pericarditis or rheumatological manifestations. The infection can be cured or develop into a chronic pulmonary disease mimicking tuberculosis. In patients with an immunodeficiency or in those who encounter a high inoculum, *Histoplasma* may spread throughout the body via the reticulo-endothelial system, and cause disseminated histoplasmosis infecting nearly all the organs.[3] The organs most often involved are liver, spleen, lymph nodes, bone marrow, adrenal glands, gastrointestinal tract and central nervous system.[2,4] As with mycobacteria, the organism can stay viable in calcified lesions. These dormant organisms can reactivate and in some cases cause systemic disease in case of waning immunity. T-cell immunity plays a paramount role in the activity of the human defence system against *Histoplasma*. T-cells and cytokines such as interleukin (IL)-12, tumour necrosis factor α. (TNF-α) and interferon-γ trigger macrophages to kill intracellular *Histoplasma*.[5]

Endemic areas for *Histoplasma* are the mid-west of the USA, the Caribbean, Central America, Africa and other tropical parts of the world.[4] The Netherlands is not a reservoir. In HIV infection, systemic infection may either be caused by exogenous exposure in endemic regions, or in nonendemic areas such as the Netherlands, by reactivation of latent infection. In nonendemic areas, clusters of cases of infection are sometimes caused by micro-foci of *Histoplasma*.[6,7] With the increasing number of immigrants from Africa and the (former) Dutch colonies in the Caribbean and Surinam, Dutch physicians are more likely to encounter cases of histoplasmosis. Data from the HIV Monitoring Foundation show that in the Netherlands all patients with histoplasmosis except one originated from endemic areas, pointing to reactivation of infection secondary to severe immunodeficiency.

The diagnosis of disseminated histoplasmosis requires knowledge of the various often nonspecific modes of presentation. Common symptoms and signs are fever (92-95%), weight loss (63-95%), diarrhoea (50%), pneumonitis (50%), lymphadenopathy (20%) and hepatosplenomegaly (25-42%).[1,8] Less commonly occurring symptoms are gastrointestinal, skin, mucosal and central nervous system abnormalities. Keys to diagnosis are laboratory findings suggesting multiorgan involvement, blood cultures (positive in 85%), chest X-ray (interstitial or reticulonodular infiltrates with or without mediastinal adenopathy in 45-70%) and cerebral fluid examination.[6,9,10] Bone marrow infection is suggested by anaemia, leucopenia and thrombocytopenia. Pancytopenia is present in 35% of cases, and cultures of bone marrow are positive in over 75%. Liver involvement is often accompanied by serum elevations of transferases (48%), alkaline phosphatase, bilirubin and LDH. Because the fatality rate is high in disseminated histoplasmosis in HIV infection, prompt diagnosis is of great importance. Blood cultures yield a sensitivity as high as 85% with AIDS.[10] However, the organism grows very slowly, as was the case in our patients. An easy method is to detect antigen or antibodies against the capsule of the fungus in urine or blood.[10-12] Sensitivity during an outbreak in Indiana was reported to be 92% for antigen tests and 71% for serology. For both the antigen and serological tests, cross-reactivity with other fungal infections hampers its use. Besides, the antibodies are often absent in case of severe immunodeficiency. The sensitivity for the antigen test is higher in urine than in serum and is especially useful in monitoring the progress of therapy.[6]

Especially LDH is a useful tool in diagnosis. In a study by Corcoran and coworkers, AIDS patients with disseminated histoplasmosis were found to have an average LDH of 1356 IU/l compared with 332 IU/l in patients with other pulmonary processes. LDH levels were more than 600 IU/l in 73% of patients with disseminated histoplasmosis compared with 10% of controls ($p<0.001$).[13] A descriptive study of patients in an endemic area found elevated LDH levels of >3 times the normal level in 74% of patients.[1] Other studies have also identified a high LDH as a positive predictor for histoplasmosis, especially if there are differential diagnostic problems with mycobacterial disease and *Pneumocystis pneumonia*.[14-17]

Furthermore, a high LDH is associated with a fatal outcome in disseminated histoplasmosis.[18] The high enzyme levels might have their origin in the bone marrow where they are formed in a process called haemophagocytosis or the reactive haemophagocytic syndrome.[17,18] One study indeed found an association between high serum LDH levels and bone marrow stain positivity for histoplasmosis.[16] Haemophagocytosis is due to inappropriate monocyte activation associated with a number of infections, immune diseases and malignancies. Its characteristics are high fever, anaemia, coagulation disorders, hypotension, liver dysfunction and systemic proliferation of mature histiocytes showing haemophagocytosis. Microscopically, histiocytes can be seen with an abundant load of intracytoplasmic *Histoplasma* and phagocytosed normoblasts. The incidence in disseminated histoplasmosis has been observed to be as high as 67%.[17] To our knowledge, no research has been done on the predominant isoforms of the high LDH levels. In case report 4, the LDH isoenzymes were measured by electrophoresis. All iso-enzymes seemed to be elevated in the patient's serum, with the elevation of LD_3 (H_2M_2 tetramere type) being most pronounced. The chemical patterns of the iso-enzymes are therefore nonspecific, but resemble those in mononucleosis, myeloid leukaemia, carcinomatosis and pancreatitis. Further research is needed to the origin of the LDH. Possible aetiological processes include liver damage, haemophagocytosis and general tissue destruction by massive dissemination.

Histology is of highest importance to the diagnosis. In tissue samples, typical intracellular budding yeast can be observed within the macrophages with a periodic acid Schiff (PAS) or Grocott stain. The liver is almost always infected, making it a good target organ for biopsy.

Untreated, acute disseminated histoplasmosis in immunosuppressed patients is fatal.[19,20] With treatment, the mortality can be decreased to less than 25%.[20] The Guidelines of the Infectious Disease Society of America-Mycoses Study Group (IDSA-MSG) might well be considered the gold standard for treatment.[20] Therapy is divided into a 12-week induction phase and, in case of immunodeficiency, a lifelong maintenance phase to prevent relapse. A recent study suggested that maintenance therapy might be safely discontinued after successful treatment with highly active anti-retroviral therapy (HAART).[21] Due to the small number of studied subjects, the IDSA still advises lifelong prophylaxis to prevent relapse. Amphotericin B is recommended for induction, followed by itraconazole 200 mg twice daily when possible. As an alternative, itraconazole can be given to less ill patients. Fluconazole, though less efficacious, is an alternative for patients intolerant to itraconazole. Recently, posaconazole was suggested as a rescue treatment modality after its efficacy against histoplasmosis had been proven in animal models.[22] Another promising but even less studied agent is voriconazole.

Many investigators, for instance the Swiss HIV Cohort Study Group and the HIV Outpatient Study Investigators (USA), have demonstrated a decrease in AIDS-related diagnoses with HAART.[23-25] However, to our knowledge, large studies on the effect of HAART on the prevalence and treatment of disseminated histoplasmosis are not available. Since disseminated histoplasmosis usually occurs with a CD4 count of less than 100,[1] the use of potent antiretroviral therapy is likely to decrease the incidence of disseminated histoplasmosis.

CONCLUSION

In Northwest European HIV patients originating from the Antilles, Surinam and other endemic areas, fever and a high LDH should raise suspicion of disseminated histoplasmosis, especially in the presence of retinal fungal infection. Liver biopsy has a high diagnostic yield. LDH might be used to treat histoplasmosis empirically before definite identification of the organism is available. Between 2000 and 2006, only 14 patients with HIV were diagnosed with histoplasmosis in the Netherlands. Disseminated histoplasmosis is a treatable HIV complication if recognised and treated early and has an excellent prognosis with antiviral treatment and secondary itraconazole prophylaxis. The low incidence and growing number of patients at risk make a higher awareness of the disease and its symptoms in nonendemic areas critically important.

REFERENCES

1. Gutierrez ME, Canton A, Sosa N, Puga E , Talavera L. Disseminated histoplasmosis in patients with AIDS in Panama: A review of 104 cases. Clin Infect Dis 2005;40:1199-202.
2. Medoff G, Kobayashi GS. Systemic mycoses caused by primary pathogens. In: Schaechter M Medoff G Eissenstein BI (eds). Mechanisms of microbial disease. 2nd ed. Baltimore, Maryland, USA: Williams & Wilkins; 1993. p. 569.
3. Wheat LJ. Histoplasmosis: recognition and treatment. Clin Infect Dis 1994;19(suppl 1):S19-27.
4. Bennett JE. Histoplasmosis. In: Braunwald et al., editor. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill; 2001. p. 1171-2.
5. Zhou P, Sieve MC, Tewari RP, Seder RA. Interleukin-12 modulates the protective immune response in SCID mice infected with Histoplasma capsulatum. Infect Immun 1997;65(3):936-42.
6. Wheat LJ, Connolly-Stringfield PA, Baker RL, et al. Disseminated Histoplasmosis in the Acquired Immune Deficiency Syndrome: Clinical findings, diagnosis and treatment, and review of literature. Medicine 1990;69(9):361-74.
7. Keath EJ, Kobayashi GS, Medoff G. Typing of Histoplasma capsulatum by restriction fragment length polymorphisms in a nuclear gene. J Clin Microbiol 1992;30(8):2104-7.

8. Wheat J. Histoplasmosis in the acquired immunodeficiency syndrome. Curr Top Med Mycol 1996;7:7-18.

9. Conces DJ Jr, Stockberger SM, Tarver RD, Wheat LJ. Disseminated histoplasmosis in AIDS: findings on chest radiographs. Am J Roentgenol 1993;160(1):15-9.

10. Williams B, Fojtasek M, Connolly-Stringfield P, Wheat J. Diagnosis of histoplasmosis by antigen detection during an outbreak in Indianapolis, Ind. Arch Pathol Lab Med 1994;118:1205-8.

11. Fojtasek MF, Kleiman MB, Connolly-Stringfield P, Blair R, Wheat LJ. The Histoplasma capsulatum antigen assay in disseminated histoplasmosis in children. Pediatr Infect Dis J 1994;13(9):801-5.

12. Wheat LJ, Kohler RB, Tewari RP. Diagnosis of disseminated histoplasmosis by detection of Histoplasma capsulatum antigen in serum and urine specimens. N Engl J Med 1986;314(2):83-8.

13. Corcoran GR, Al-Abdely H, Flanders CD, Geimer J, Patterson TF. Markedly elevated serum lactate dehydrogenase levels are a clue to the diagnosis of disseminated histoplasmosis in patients with AIDS. Clin Infect Dis 1997;24(5):942-4.

14. Butt AA, Michaels S, Greer D, Clark R, Kissinger P, Martin DH. Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS Read 2002;12(7):317-21.

15. Graviss EA, Vanden Heuvel EA, Lacke CE, Spindel SA, White AC Jr, Hamill RJ. Clinical prediction model for differentiation of disseminated Histoplasma capsulatum and Mycobacterium avium complex infections in febrile patients with AIDS. J Acquir Immune Defic Syndr 2000;24(1):30-6.

16. Luther JM, Lakey DL, Larson RS, et al. Utility of bone marrow biopsy for rapid diagnosis of febrile illnesses in patients with human immunodeficiency virus infection. South Med J 2000;93(7):692-7.

17. Koduri PR, Chundi V, DeMarais P, Mizock BA, Patel AR, Weinstein RA. Reactive hemophagocytic syndrome: a new presentation of disseminated histoplasmosis in patients with AIDS. Clin Infect Dis 1995;21(6):1463-5.

18. Couppié P, Sobesky M, Aznar C, et al. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin Infect Dis 2004;38(1):134-8.

19. Goodwin RA Jr, Shapiro JL, Thurman GH, Thurman SS, des Prez RM. Disseminated histoplasmosis: Clinical and pathologic correlations. Medicine (Baltimore) 1980;59:1-33.

20. Wheat J, Sarosi G, McKinsey D, et al. Practice guidelines for the management of patients with histoplasmosis. Infectious Diseases Society of America. Clin Infect Dis 2000;30(4):688-95.

21. Goldman M, Zackin R, Fichtenbaum DJ, et al. Safety of discontinuation of maintenance therapy for disseminated histoplasmosis after immunologic response to antiretroviral therapy. Clin Infect Dis 2004;38:1485-9.

22. Clark B, Foster R, Tunbridge A, Green S. A case of disseminated histoplasmosis successfully treated with the investigational drug posaconazole. J Infect 2005;51(3):177-80.

23. Koletar SL, Willaims PG, Wu J, et al. Long-term follow-up of HIV-infected individuals who have significant increases in CD4+ cell counts during antiretroviral therapy. Clin Infect Dis 2004;39:1500-6.

24. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338(13):853-60.

25. Ledergerber B, Egger M, Erard V, et al. AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy. The Swiss HIV Cohort Study. JAMA 1999;282(23):2220-6.

Netherlands
The Journal of Medicine

CASE REPORT

Sporadic porphyria cutanea tarda due to haemochromatosis

H.R.H. de Geus[1*], A. Dees[2]

[1]Department of Intensive Care, Erasmus Medical Centre, Rotterdam, the Netherlands (present address),
[2]Department of Internal Medicine, Ikazia Hospital, Rotterdam, the Netherlands,
[*]corresponding author: e-mail: geushrhde@yahoo.com

ABSTRACT

Haemochromatosis is a hereditary iron-overload syndrome caused by increased intestinal iron absorption and characterised by accumulation of potentially toxic iron in the tissues. Sometimes this disease presents as a cutanea porphyria. We describe a patient with joint complaints and blistering skin lesions on sun-exposed skin. After identifying the porphyria cutanea tarda by urine analysis we found that the serum activity of uroporphyrinogen decarboxylase (UROD) was normal, meaning a partial inactivation of UROD in liver tissue due to external factors. Further investigation showed the homozygous Cys282Tyr missense mutation and high levels of serum ferritin. It is important to recognise the symptoms of iron overloading at an early stage because hereditary haemochromatosis needs to be treated immediately. We therefore advocate routine sampling of ferritin levels in patients with unexplained joint complaints.

KEYWORDS

Arthralgias, blistering skin lesions, ferritin, haemochromatosis, porphyria cutanea tarda

INTRODUCTION

Haemochromatosis is an iron-overload syndrome caused by increased intestinal absorption and characterised by accumulation of potentially toxic iron in the tissues. There are primary genetic forms expressed in adults (the Cys282Tyr and H63D mutations) and hereditary juvenile and neonatal forms. Secondary haemochromatosis can be a result of excessive intake of iron, augmented by the use of alcohol and vitamin C, and of multiple blood transfusions, usually seen in patients with persistent anaemias. In its early stages systemic iron deposition leads to mild symptoms such as fatigue, arthralgias and arthrosis. Untreated though, it can result in major organ damage such as congestive heart failure, cardiac arrhythmias, bronze diabetes, hepatic cirrhosis, impotence and infertility.[1] Early recognition of the symptoms is of crucial importance to prevent further tissue destruction of the various organ systems. The direct or indirect effect of prolonged iron overloading on the skin is the first sign of haemochromatosis. We present a patient referred because of untreatable arthralgias of the hands and blistering lesions on sun-exposed skin.

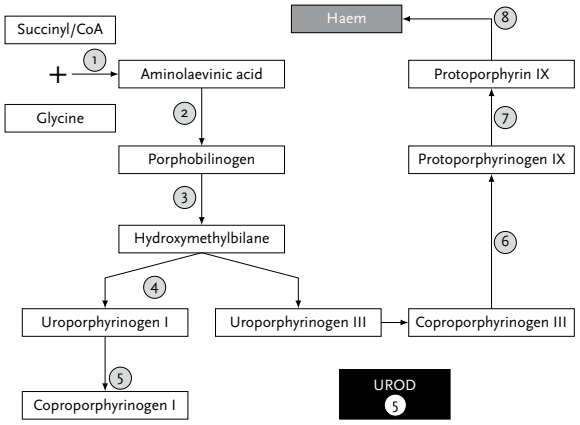
CASE REPORT

A 56-year-old male presented with arthralgias, blistering skin lesions on sun-exposed skin and slightly raised liver enzymes: bilirubin 18 μmol/l (0-17), aspartate aminotransferase 75 U/l (<31), alanine aminotransferase 122 U/l (<31), and γ-glutamyltransferase 75 U/l (<35). He consumed two glasses of wine a day. The patient did not have any close relatives such as children, brothers or sisters. Physical examination did not reveal any signs of arthritis or an enlarged liver. However, remarkable blistering skin lesions on his hands and lower legs were seen. Ultrasound of the liver suggested steatosis hepatis without focal lesions. Hepatitis B/C virus, Epstein-Barr virus and cytomegalovirus serology were normal, as were serum levels of the rheumatoid factors. The differential diagnosis consisted of porphyria cutanea tarda (PCT) or parapemphigus. Subsequent 24-urine analysis revealed elevated levels of uroporphyrinogen III at 116 nmol/mmol creatinine (<2.0), hepta-carboxyl porphyrin 72 nmol/mmol creatinine (<1.6), hexa-carboxyl porphyrin 8.0 nmol/mmol creatinine (<2.4) and penta-carboxyl porphyrin 20 nmol/mmol creatinine (<0.5), indicating partial blockage of the

decarboxylation of uroporphyrinogen III. The activity of uroporphyrinogen decarboxylase (UROD) in erythrocytes, which was normal, differentiated between an acquired and inherited subtype of PCT. To confirm the diagnosis of sporadic type I PCT due to iron overload in case of haemochromatosis, we tested ferritin levels in plasma (>1450 µg/l), iron saturation (105.5%) and HFE gene analysis. This gene turned out to be homozygously changed with the Cys282Tyr missense mutation. A liver biopsy was not performed, considering the highly invasive nature of this test without any clinical consequences, because there were no radiological and biochemical signs of fibrosis.

Our patient was strongly advised to stop the use of alcohol-containing products and repeatedly underwent phlebotomy, resulting in a decrease in the ferritin levels of up to 150 µg/l with acceptable haemoglobin levels. The skin abnormalities disappeared rapidly and his joint complaints were controllable with non-steroidal anti-inflammatory drugs. Outpatient visits were continued to control the ferritin levels and six-monthly serum tests of α-fetoprotein levels (AFP) will be performed because of the increased risk of hepatocellular carcinoma in this patient.

Figure 1. *The biosynthetic pathway of haem*



Eight different enzymes catalyse the formation of haem: 1) aminolaevulinic acid synthetase, 2) aminolaevulinic acid dehydratase, 3) porphobilinogen deaminase, 4) uroporphyrinogen III cosynthase, 5) uroporphyrinogen decarboxylase (UROD), 6) coproporphyrinogen oxidase, 7) protoporphyrinogen oxidase, 8) ferrochelatase.

DISCUSSION

In the patient described above, hereditary haemochromatosis was found with a mutation in the HFE gene. Why were iron overload and skin blistering related here? The key to this question is incomplete haemsynthesis, producing toxic precursors. In our patient, the blockage in the haem pathway was found at the level of UROD which decarboxylates uroporphyrinogen III to coproporphyrinogen III in four steps producing hepta-, hexa- and penta-carboxyl porphyrin as by-products (*figure 1*; decarboxylation by-products are not shown). The effects of iron on the formation of haem precursors are thought to consist of three cornerstones. First, iron catalyses the formation of reactive oxygen molecules enhancing uroporphyrin formation, secondly iron enhances the production of nonporphyrin products directly inhibiting UROD and thirdly iron induces δ-aminolaevulinic acid synthetase and thus the production of δ-aminolaevulinic acid, the precursor of uroporphyrinogen.[2]

Deficiency or inactivation of UROD causes accumulation and skin deposition of the haem precursor uroporphyrinogen. This molecule is potentially toxic to our skin because of its photosensibility for ultraviolet light (400 nm). The excited energy state leads to complement activation and release of histamine resulting in blistering skin lesions. Expression of this sporadic subtype of PCT (s-PCT) is usually observed in the third or fourth decade, since substantial accumulation of iron is required to successfully block the pathway. Hepatitis C infection, alcohol use and usage of oestrogens contribute to the expression of s-PCT.[3,4] Combining these risk factors with hereditary or secondary derived haemochromatosis predisposes even more to the expression of s-PCT.[5,6]

Buljai *et al.* performed a study to investigate the correlation of hereditary haemochromatosis (HH) and sporadic porphyria cutanea tarda in an American patient population; they found a significantly higher incidence of s-PCT in homozygous HH patients compared with heterozygous patients and nonaffected controls.[7]

The performance of liver biopsies used to be considered the gold standard in diagnosing haemochromatosis. With modern gene analysis, diagnosing HH is much easier and liver biopsies are only performed for prognostic reasons because of potential cirrhosis and hepatocellular carcinoma (HCC). Furthermore, several authors have shown that with simple clinical and biochemical variables (ferritin, platelet counts and aspartate aminotransferase values, absence of hepatomegaly) an accuracy of 77 to 90% can be reached for correctly diagnosing the absence of fibrosis, thus reducing the need for liver biopsy.[8,9] Organ-specific imaging techniques such as magnetic resonance imaging (MRI) will be used in the evaluation of the HH patient in near future. Monitoring is of growing importance, since there is a 20-fold increased risk of developing HCC, especially in men. The highest risk of developing HCC is found in patients with established cirrhosis, thus screening should be focused in that direction.

As in our patient, the recognition of the skin lesions led to the final diagnosis of hereditary haemochromatosis. In retrospection, he had a history of nonspecific joint complaints for two years without any significant skin lesions. Rheumatologists in our country usually perform routine blood tests containing ferritin sampling on patients with arthralgias. Whether or not this is cost-effective in screening for HH in patients with joint complaints is unknown, especially because of the low sensitivity and specificity of the test. The issue remains that we probably would have diagnosed our patient with HH in an earlier stage if we had performed a ferritin sample because of his joint complaints on his first visit to the outpatient clinic.

REFERENCES

1. Matthews AL, Grimes SJ, Wiesner GL, Acheson LS. Clinical consult: iron overload--hereditary hemochromatosis. Prim Care 2004;31(3):767-xiii.
2. Lambrecht RW, Bonkovsky HL. Hemochromatosis and porphyria. Semin Gastrointest Dis 2002;13(2):109-19.
3. Roenigk HH Jr, Gottlob ME. Estrogen-induced porphyria cutanea tarda. Report of three cases. Arch Dermatol 1970;102(3):260-6.
4. Bonkovsky HL, Poh-Fitzpatrick M, Pimstone N, et al. Porphyria cutanea tarda, hepatitis C, and HFE gene mutations in North America. Hepatology 1998;27(6):1661-9.
5. Cruz-Rojo J, Fontanellas A, Moran-Jimenez MJ, et al. Precipitating/aggravating factors of porphyria cutanea tarda in Spanish patients. Cell Mol Biol (Noisy-le-grand) 2002;48(8):845-52.
6. Tannapfel A, Stolzel U, Kostler E, et al. C282Y and H63D mutation of the hemochromatosis gene in German porphyria cutanea tarda patients. Virchows Arch 2001;439(1):1-5.
7. Bulaj ZJ, Phillips JD, Ajioka RS, et al. Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda. Blood 2000;95(5):1565-71.
8. Beaton M, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. Hepatology 2002;36(3):673-8.
9. Guyader D, Jacquelinet C, Moirand R, et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. Gastroenterology 1998;115(4):929-36.

Netherlands
The Journal of Medicine

CASE REPORT

Diabetes insipidus and adrenal insufficiency in a patient with metastatic breast cancer

T. Netelenbos[1*], M.A. Nooij[2], J.W.R. Nortier[2]

Departments of [1]Internal Medicine and [2]Clinical Oncology, Leiden University Medical Centre, Leiden, the Netherlands, [*]corresponding author: tel.: +31 (0)71-526 30 57, fax: +31 (0)71-526 68 81, e-mail: T.Netelenbos@lumc.nl

ABSTRACT

A patient previously treated for bilateral breast cancer with mastectomy, radiation therapy and in remission on hormonal therapy for more than five years presented with abdominal symptoms from breast cancer relapse. She developed inappropriate polyuria and hypernatraemia, which responded to desmopressin. In combination with the absence of a high signal from the posterior lobe of the pituitary on MRI, these data indicated the presence of partial central diabetes insipidus. The anterior pituitary showed partial failure (low follicle-stimulating hormone, luteinising hormone and insulin-like growth factor-1 levels). Furthermore, primary adrenal insufficiency had developed, ascribed to bilateral tumour invasion of the adrenals. This rare combination of endocrinological failures in a patient with metastatic breast cancer is discussed.

KEYWORDS

Breast cancer, central diabetes insipidus, primary adrenal insufficiency

INTRODUCTION

Endocrine syndromes can occur as a result of tumour metastases. Diabetes insipidus has been described in patients with advanced breast cancer, although in general clinical manifestations of metastases due to involvement of the hypothalamic-pituitary region are rare.[1] Adrenal metastases occur frequently, but only rarely cause adrenal failure.[2] We present a 57-year-old patient with breast cancer who developed partial central diabetes insipidus (CDI), partial anterior pituitary failure and primary adrenal insufficiency from metastases.

CASE REPORT

A 57-year-old woman presented in 2004 with a two-month history of abdominal pain, dysphagia and occasional vomiting. Her medical history revealed bilateral breast cancer in 1997, for which radical mastectomy and bilateral axillary dissection were performed. Histological examination showed bilateral infiltrating ductal carcinoma and bilateral axillary node metastases; a left supraclavicular lymph node also showed tumour infiltration (left breast: pT1N1M1, right breast: pT1N1M0). The oestrogen receptor was weakly positive for both tumours and the progesterone receptor was weakly positive for the left and positive for the right breast. In addition, she was treated with bilateral locoregional radiotherapy including the left supraclavicular lymph node metastasis and started on systemic adjuvant endocrine therapy with tamoxifen. In 1998 she developed lymphangitis of the chest wall. Subsequently, the hormonal therapy was switched to anastrozole and she attained a complete remission for five and a half years.

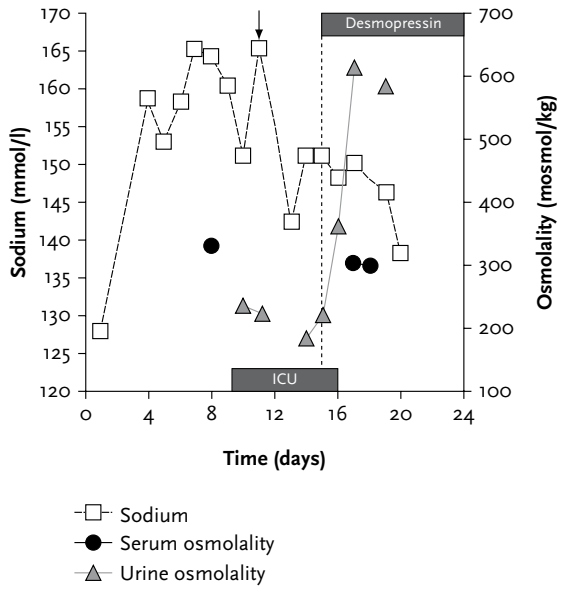
Evaluation of her abdomen by a computed tomography (CT) scan showed bilateral hydronephrosis and extensive retroperitoneal, mesenterial and para-aortal lymphadenopathy, as well as bilateral large adrenal glands (right: 41 x 21, left: 44 x 24 mm). Double J stents were inserted. Biopsy from one of the adrenal masses revealed infiltrative ductal carcinoma (oestrogen receptor positive with weak expression of progesterone receptor). Overexpression of HER2 could not be detected on the biopsy specimen nor on either of the primary tumours. She started palliative chemotherapy consisting of cyclophosphamide and doxorubicin. Six days later, she was admitted to our hospital with dysphagia and vomiting, but was still able to drink. She had lost three kilograms in weight. On physical examination a tired looking woman was seen with a body weight of 67 kg. Blood pressure was 100/60 mmHg, pulse rate 68 beats/min and body temperature 37.4 °C. Apart from scars from

the bilateral mastectomy there were no abnormalities on physical examination. Laboratory investigations showed the following results: haemoglobin 7.0 mmol/l (7.5-10.0), mean cell volume 89 fl (80-100), leucocytes 1.6 x 10^9/l (4.5-10) with 81.3% neutrophils, thrombocytes 221 x 10^9/l (150-450), sodium 128 mmol/l (136-144), potassium 3.7 (3.6-4.8), urea 10.0 mmol/l (2.5-7.5), creatinine 106 µmol/l (<80), albumin 39 g/l (40-50), and calcium 2.45 mmol/l (2.25-2.55 mmol/l).

Three days after admission she developed fever with neutropenia. One blood culture revealed *Escherichia coli*, whereas urine cultures were negative. Ultrasound examination of the abdomen showed persistent hydronephrosis of the right kidney. The right double J stent was therefore exchanged. After four days she developed hypernatraemia with a highest value of 165 mmol/l *(figure 1)* and hypokalaemia (2.5 mmol/l). She admitted being thirsty. One week after admission, she became polyuric (3.5-5.5 litres/day) and somnolent and the suspicion of diabetes insipidus arose. Her medical condition deteriorated and she had to be transferred to the intensive care unit because of haemodynamic instability and fear of respiratory arrest. During her stay in intensive care, she required mechanical ventilation for four days and was persistently polyuric and hypernatraemic *(figure 1)* in the presence of inappropriately low urine osmolality. Desmopressin resulted in a reduction in the polyuria associated with an increase in urine osmolality (*figure 1*). Because of persistently low blood pressures, an adrenocorticotropic hormone (ACTH) stimulation test was performed. This test showed a basal cortisol level of 0.29 µmol/l with a maximal increase of cortisol to only 0.41 µmol/l on ACTH administration (0.25 mg, *table 1*), indicating primary adrenal failure. Therefore hydrocortisone was started (100 mg three times daily).

After returning to the oncology ward, she gradually recovered from her somnolent state. She then told us that she had been drinking about four litres of fluid a day since 1997, also during the night time. Magnetic resonance imaging (MRI) of the brain with gadolinium enhancement revealed no visible tumours. However, the posterior lobe of the pituitary did not reveal the usual enhanced signal, suggesting tumour infiltration. Hormonal evaluation of other pituitary axes showed low levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH), despite her postmenopausal status (*table 2*). The ACTH stimulation test was repeated as the first test was carried out under high stress conditions (after cessation of hydrocortisone), and showed a normal basal cortisol level in the presence of elevated basal ACTH (584 ng/l) and an absent cortisol response (*table 1*). Insulin-like growth factor-1 (IGF-1) levels were low compared with age- and

Figure 1. *Time course of serum sodium levels and osmolality in serum and urine*



Time is depicted in days after admission. The arrow marks the first gift of desmopressin intravenously (2 x 2 µg), which was given continuously from day 14 onwards. The intensive care unit (ICU) stay is marked.

Table 1. *Results from ACTH stimulation tests*

	Cortisol (µmol/l)			ACTH (<75 ng/l)
	t = 0	t = 30	t = 60	t = 0
Day 12	0.29	0.41	0.38	
Day 35	0.51	0.53	0.52	584

Days are counted from admission. t = time in minutes after administration of 0.25 mg ACTH. Normal ACTH values are given in parentheses.

Table 2. *Laboratory results from anterior pituitary hormones*

	Day 18	Day 27	Day 41	N
FT4	7.7	14.4		10-24 pmol/l
TSH	2.36	5.62		0.3-4.8 mU/l
LH	0.9	6.8	7.2	11-75 U/l
FSH	6.3	17	14	>30 U/l
Prolactin	13	25		<30 µg/l
GH	0.75			0-5 mU/l
IGF-1	7.8	5.3		11.6-48.4 nmol/l

Days are counted from admission. N = normal values; FT4 = free thyroxin; TSH = thyroid stimulating hormone; LH = luteinising hormone; FSH = follicle-stimulating hormone; IGF-1 = insulin-like growth factor-1; GH = growth hormone.

gender-matched values. This could be explained by her clinical condition and/or growth hormone (GH) deficiency. Because of the contraindications for GH replacement (i.e. presence of cancer), we did not perform a GH stimulation test to confirm a diagnosis of GH deficiency.

Firstly, it was concluded from the clinical symptoms, laboratory results and MRI that she had a partial central diabetes insipidus, possibly present for several years, most likely caused by breast cancer metastases. In addition, she had partial anterior pituitary failure, reflected in low FSH, LH and IGF-1 (*table 2*). Secondly, she had bilateral adrenal gland metastases leading to primary adrenal insufficiency under stressful clinical conditions. She was treated with hydrocortisone in a substitution dose (20-10-10 mg daily) and desmopressin nasal spray 10 μg twice daily, after which the sodium levels returned to normal and the polyuria diminished.

Follow-up in the outpatient department showed a regression of the lymphadenopathy and reduction in the volume of adrenal gland metastasis on abdominal CT scan after six cycles of cyclophosphamide and doxorubicin.

DISCUSSION

Diabetes insipidus is a syndrome characterised by hypotonic polyuria and polydipsia, either as a result of inadequate antidiuretic hormone (ADH) secretion (central diabetes insipidus (CDI)), inadequate renal response to ADH (nephrogenic diabetes insipidus) or primary polydipsia. Causes of CDI are congenital or acquired lesions that disrupt the neurons that originate in the supraoptic and paraventricular nuclei of the hypothalamus axis. These lesions are malformations, damage resulting from surgery or trauma, tumours, haemorrhage, thrombosis, infarction or granulomatous disease. Some 30 to 50% of cases are idiopathic.[3,4] Primary tumours are craniopharyngioma, meningioma or germinoma, but secondary tumours can also occur. Metastases in the posterior pituitary lobe are more common than in the anterior lobe, possibly caused by the direct blood supply of the posterior lobe from the systemic circulation. Metastases in the hypothalamic-pituitary region at autopsy are relatively common in breast cancer, varying from 5.3 to 28%.[1,5] However, clinical symptoms of CDI in patients with metastatic breast cancer are rare.

On MRI the posterior pituitary is identified by hyperintensity, probably caused by phospholipids or secretory granules in pituicytes.[6] A lack of this hyperintensity on sagittal T1-weighted images, as was observed in our patient, is the hallmark of hypothalamic-posterior pituitary disorders and may represent an early stage of tumour infiltration.[4,7] A thickened pituitary stalk could be another indicative finding.

Our patient developed hypernatraemia and hyperosmolarity while producing many litres of urine of a low osmolality (*figure 1*), probably triggered by insufficient water intake. There was no glucosuria or other causes of osmotic diuresis. A water deprivation test was not performed because of the high clinical suspicion of central diabetes insipidus, the instable condition of the patient and the good response to desmopressin, which indicates insufficient endogenous ADH secretion. Our patient stated that she had been drinking many litres a day for years, suggesting that she had pituitary metastases while being in complete remission for five years on anastrozole.

All anterior pituitary gland hormones could be involved in the metastatic process. In our patient serum gonadotropin levels were inappropriately low for a postmenopausal woman. In contrast to tamoxifen, which reduces gonadotropin levels by its weak oestrogen agonistic effect, anastrozole, an aromatase inhibitor, is expected to increase FSH and LH.[8] Furthermore, IGF-1 levels, controlled by GH, were also low. These findings are in accordance with the diagnosis of partial pituitary insufficiency. The abnormalities found in free thyroxin and thyroid-stimulating hormone could be ascribed to nonthyroidal illness, as these levels returned to normal values.

Primary adrenal insufficiency is usually caused by autoimmune disease. Other causes are infections or haemorrhagic infarction of the adrenals. Infiltration of the adrenal glands by metastases (lung, breast, melanoma, lymphoma) is common, but this rarely leads to adrenal insufficiency, probably because not all cortex tissue is destroyed.[2,9] However, there are reports that patients with bilateral adrenal gland metastases are prone to develop partial adrenal insufficiency, requiring corticosteroid replacement therapy.[10,11]

During critical illness and stress situations, such as in intensive care units, relative adrenal insufficiency can be caused by maximal endogenous cortisol stimulation. An increase in cortisol after exogenously administered ACTH can than be absent, as was found in the first ACTH test in our patient.[12] The second test showed a higher basal cortisol level, but in the presence of increased ACTH levels and no response to exogenous ACTH administration, indicating primary hypoadrenalism.[13,14] Probably the combination of illness together with adrenal gland tumour invasion led to (subclinical) adrenal insufficiency.

The serum electrolyte abnormalities known to be caused by adrenal insufficiency are in contrast to those found in our patient. This can be caused by diminished glucocorticoid production rather than mineralocorticoid deficiency and by the simultaneously occurring hypernatraemic CDI. The hyponatraemia associated with adrenal insufficiency is caused by volume contraction and inappropriate ADH

secretion[15] and upregulation of aquaporin-2 (the water channels regulated by ADH).[16] As ADH production could not be increased, hyponatraemia did not occur in our patient. Another interaction between ADH and the hypothalamus-pituitary-adrenal gland axis is the association of CDI with higher ACTH and cortisol levels by as yet unclear mechanisms.[17,18] On the other hand, secondary adrenal insufficiency results in higher ADH levels.[19] Mineralocorticoid treatment was not started because of the good clinical response to hydrocortisone alone and the possibility of electrolyte disturbances from the simultaneously occurring CDI and hypokalaemia.
Our patient needed potassium chloride suppletion during her hospital stay. She received parenteral feeding for several weeks because oral feeding resulted in vomiting and subileus, probably due to the intra-abdominal metastases. The loss of potassium was likely caused by gastric secretions and renal loss from the polyuria. After correction of the potassium, polyuria persisted. Short-lived nephrogenic diabetes insipidus can result from hypokalaemia, but the mild concentrating defect does not cause hyperosmolality and responds to correction. This probably results from downregulation of aquaporin-2 and does not respond to desmopressin.[20]

The combination of failure in the hypothalamic-pituitary area and primary adrenal insufficiency is rarely reported.[21] Trincado *et al*. described a male patient with bronchogenic carcinoma whose first clinical manifestation was diabetes insipidus secondary to metastases to the hypothalamic-pituitary area who developed anterior pituitary failure, as well as primary adrenal insufficiency due to metastases in both adrenals.

In conclusion, we present a patient with breast cancer relapse after having been in remission for more than five years on hormonal therapy, who developed partial CDI, partial anterior pituitary insufficiency and primary adrenal insufficiency.

ACKNOWLEDGEMENT

The authors would like to thank Professor J.A. Romijn for his critical reading of the manuscript.

REFERENCES

1. Huinink DT, Veltman GA, Huizinga TW, Roelfsema F, Keizer HJ. Diabetes insipidus in metastatic cancer: two case reports with review of the literature. Ann Oncol 2000;11(7):891-5.
2. Lutz A, Stojkovic M, Schmidt M, Arlt W, Allolio B, Reincke M. Adrenocortical function in patients with macrometastases of the adrenal gland. Eur J Endocrinol 2000;143(1):91-7.
3. Verbalis JG. Management of disorders of water metabolism in patients with pituitary tumors. Pituitary 2002;5(2):119-32.
4. Maghnie M. Diabetes insipidus. Horm Res 2003;59(suppl 1):42-54.
5. Fassett DR, Couldwell WT. Metastases to the pituitary gland. Neurosurg Focus 2004;16(4):E8.
6. Shin JH, Lee HK, Choi CG, et al. MR imaging of central diabetes insipidus: a pictorial essay. Korean J Radiol 2001;2(4):222-30.
7. Chaudhuri R, Twelves C, Cox TC, Bingham JB. MRI in diabetes insipidus due to metastatic breast carcinoma. Clin Radiol 1992;46(3):184-8.
8. Bajetta E, Zilembo N, Bichisao E, Pozzi P, Toffolatti L. Steroidal aromatase inhibitors in elderly patients. Crit Rev Oncol Hematol 2000;33(2):137-42.
9. Lam KY, Lo CY. Metastatic tumours of the adrenal glands: a 30-year experience in a teaching hospital. Clin Endocrinol (Oxf) 2002;56(1):95-101.
10. Redman BG, Pazdur R, Zingas AP, Loredo R. Prospective evaluation of adrenal insufficiency in patients with adrenal metastasis. Cancer 1987;60(1):103-7.
11. Seidenwurm DJ, Elmer EB, Kaplan LM, Williams EK, Morris DG, Hoffman AR. Metastases to the adrenal glands and the development of Addison's disease. Cancer 1984;54(3):552-7.
12. Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. N Engl J Med 2003;348(8):727-34.
13. Dorin RI, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. Ann Intern Med 2003;139(3):194-204.
14. Oelkers W. Adrenal insufficiency. N Engl J Med 1996;335(16):1206-12.
15. Ahmed AB, George BC, Gonzalez-Auvert C, Dingman JF. Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. J Clin Invest 1967;46(1):111-23.
16. Saito T, Ishikawa SE, Ando F, Higashiyama M, Nagasaka S, Sasaki S. Vasopressin-dependent upregulation of aquaporin-2 gene expression in glucocorticoid-deficient rats. Am J Physiol Renal Physiol 2000;279(3):F502-8.
17. Pivonello R, Faggiano A, Filippella M, et al. Hypothalamus-pituitary-adrenal axis in central diabetes insipidus: ACTH and cortisol responsiveness to CRH administration. J Endocrinol Invest 2002;25(11):932-7.
18. Itagaki E, Ozawa S, Yamaguchi S, et al. Increases in plasma ACTH and cortisol after hypertonic saline infusion in patients with central diabetes insipidus. J Clin Endocrinol Metab 2001;86(12):5749-54.
19. Yatagai T, Kusaka I, Nakamura T, et al. Close association of severe hyponatremia with exaggerated release of arginine vasopressin in elderly subjects with secondary adrenal insufficiency. Eur J Endocrinol 2003;148(2):221-6.
20. Amlal H, Krane CM, Chen Q, Soleimani M. Early polyuria and urinary concentrating defect in potassium deprivation. Am J Physiol Renal Physiol 2000;279(4):F655-63.
21. Trincado P, Playan J, Acha J, et al. Adrenal failure due to metastasis both to the hypothalamic-pituitary area and the adrenals. Tumori 1996;82(4):401-4.

Netherlands
The Journal of Medicine

PHOTO QUIZ

A patient with diabetes mellitus and recurrent peristomal bleeding

E.J. van der Wouden*, B.D. Westerveld

Department of Gastroenterology, Isala Clinics, location Sophia, Zwolle, the Netherlands,
*corresponding author: tel.: +31 (0)38-424 50 00, fax: +31 (0)38-424 76 54,
e-mail: e.j.van.der.wouden@isala.nl

KEYWORDS

Diabetes mellitus, peristomal bleeding

CASE REPORT

A 75-year-old obese man presented to the Department of Gastroenterology. His medical history included a rectal amputation and colostomy 19 years ago for rectal carcinoma. Furthermore, he had been treated for diabetes mellitus type 2 for 17 years. His diabetes mellitus was complicated by micro-albuminuria and peripheral neuropathy. Finally, he was being treated for hypertension and dyslipidaemia.
During the preceding six months, he had been treated several times for recurrent peristomal bleeding. Severe bleeding from the stoma edges started spontaneously or during stoma care. Although local pressure was applied and coagulation therapy was performed several times, which stopped the bleeding, he had to be admitted twice for blood transfusions due to severe blood loss. A colonoscopy showed no abnormalities. Inspection of his stoma showed multiple peristomal varices (*figures 1* and *2*).

WHAT IS YOUR DIAGNOSIS?

See page 316 for the answer to this photo quiz.

Figure 1. *Patient's stoma with multiple purple blue varices*

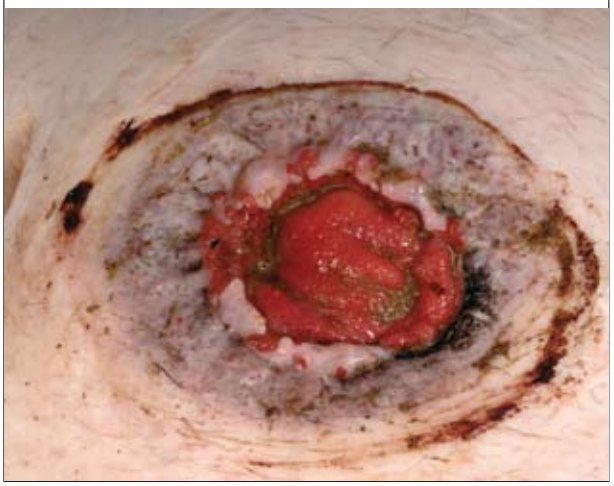
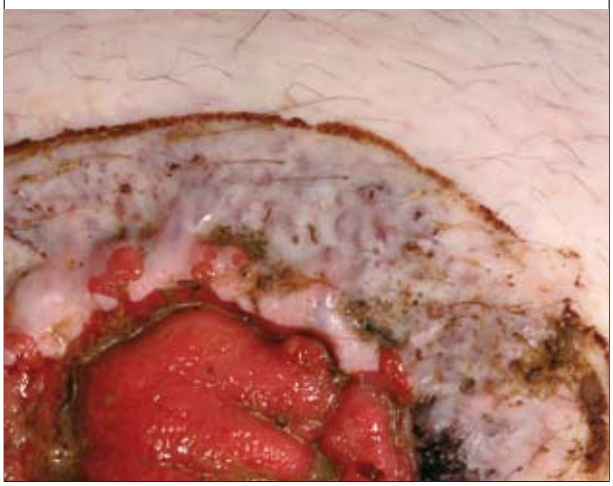


Figure 2. *Detail of the patient's stoma*



Gastro-oesophageal reflux in morbidly obese patients is associated with hiatal hernias but not with body mass index

In a recent issue of this Journal (2005;63:344-7) Loffeld concluded that there is a definite relation between body mass index (BMI) and the occurrence of gastro-oesophageal reflux disease (GERD). He also discussed the relation between obesity, GERD, and Helicobacter pylori. The exact relation and the consequences are not yet entirely clear.

As Loffeld describes, the most important pathophysiological mechanism causing reflux is long-lasting spontaneous relaxation of the lower oesophageal sphincter (LOS) or low pressure in the LOS. A hiatal hernia is an additional risk factor. Finally, increased intra-abdominal pressure plays an important role in the mechanism of reflux. Since these factors are generally accepted to be present in patients with obesity, these patients are expected to be at risk of developing GERD.

We studied the association between BMI and hiatal hernia or GERD in patients with morbid obesity. We retrospectively analysed the preoperative data of 198 morbidly obese patients (BMI >40 kg/m², or BMI > 35 kg/m² in combination with relevant comorbidity) treated by gastric banding between March 1995 and December 2000. Data of the extensive preoperative protocol were analysed for BMI, symptoms of GERD, use of PPI or H_2 blockers, and result of gastroscopy. Endoscopy was performed in 170 patients (157 females, 13 males; age 37 years, range 20 to 69 years; BMI 44.9 kg/m², range 35.6 to 60.9 kg/m²). GERD symptoms were reported in 50 patients (29.4%), eight of whom were treated by PPI or H_2 blockers. Hiatal hernias were seen in 81 patients (47.6%) and symptomatic in 30 (37.0%). Of the patients without hiatal hernia, 27.6% reported symptoms of GERD. Endoscopic signs of reflux oesophagitis were present in 61.7% of patients with hiatal hernia, vs 12.4% in those without ($p< 0.001$). There were no differences in the BMI in patients with and without GERD symptoms (44.9 ± 5.2 kg/m² vs 45.0 ± 5.8 kg/m²).

We concluded that in morbidly obese patients, GERD symptoms occur independent of BMI, but are related to the presence of hiatal hernia. Nevertheless, based on our findings, overweight cannot be excluded as a risk factor for GERD, since we did not compare our morbidly obese population with the general population. However, it seems that being obese or getting more obese does not increase the risk of developing GERD. Treatment of GERD in morbidly obese patients is medical.

However, treatment of obesity and especially surgical treatment of morbid obesity is relevant. The number of patients with obesity is growing and will give rise to serious health problems, such as, diabetes, hyperlipidaemia, hypertension, and obstructive sleep apnoea.[1-3] Recent follow-up studies have demonstrated that bariatric surgery resulted in long-term weight loss, and an improved lifestyle. Furthermore, a substantial majority of patients with diabetes, hyperlipidaemia, hypertension, and obstructive sleep apnoea experienced complete resolution or improvement.[2-4]

R.M. Kiewiet, A.C.M. van Vliet*

Department of Internal Medicine, Albert Schweitzer Hospital, PO Box 444, 3300 AK Dordrecht, the Netherlands, *corresponding author: tel.: +31 (0)78-654 11 11, fax: +31 (0)78-652 33 77, e-mail A.C.M.vanVliet@asz.nl

REFERENCES

1. Van Houwelingen KG, van Uffelen R, van Vliet ACM. The sleep apnoea syndromes [review]. Eur Heart J 1999;20:858-66.
2. Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery. A systematic review and meta-analysis. JAMA 2004:292:1724-37.
3. Sjöström L, Lindroos A-K, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683-93.
4. Pontiroli AE, Folli F, Paganelli P, et al. Laparoscopic gastric banding prevents type 2 diabetes and arterial hypertension and induces their remission in morbid obesity. A 4-year case-controlled study. Diabetes Care 2005;28:2703-9.

ANSWER TO PHOTO QUIZ (ON PAGE 314)

A PATIENT WITH DIABETES MELLITUS AND RECURRENT PERISTOMAL BLEEDING

DIAGNOSIS

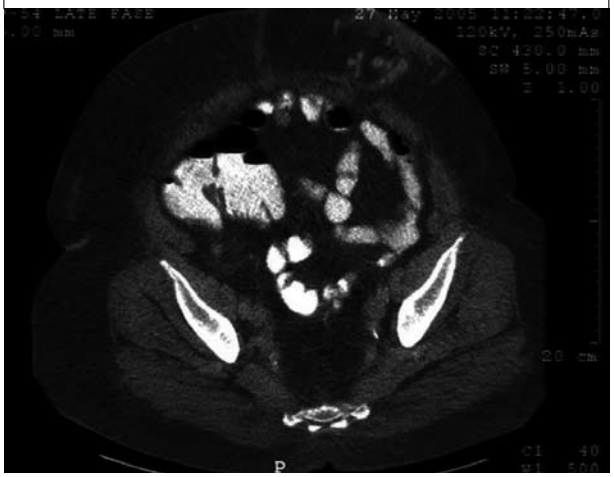
Laboratory evaluation showed a haemoglobulin of 4.6 mmol/l (8.5-11.1), MCV 79 (80-100), leucocytes 4.2 (4.0-10.0) thrombocytes 101 (150-400), a prothrombin time of 1.0 INR (0.9-1.1 INR), and an antithrombin III of 67% (80-120%). Electrolytes, renal function and liver tests were in the normal range. A gastroduodenoscopy revealed oesophageal varices grade II. A computed tomography scan of the abdomen showed an enlarged vena mesenterica with aberrant veins running to the stoma (*figure 3*). Furthermore, large aberrant veins from the stoma were running through the subcutis to the vena iliaca. The portal vein was not occluded. A diagnosis of portal hypertension was made, probably from liver cirrhosis. Further evaluation revealed no cause for the liver cirrhosis. His medical history of diabetes mellitus type 2, obesity and dyslipidaemia suggested cryptogenic liver cirrhosis due to nonalcoholic fatty liver disease. A liver biopsy was not performed.[1]

The patient was treated with propranolol and sclerotherapy of the peristomal varices.[2,3] So far, no recurrent bleeding has occurred.

REFERENCES

1. Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705-25.
2. Wolfsen HC, Kozarek RA, Bredfeldt JE, Fenster LF, Brubacher LL. The role of endoscopic injection sclerotherapy in the management of bleeding peristomal varices. Gastrointest Endosc 1990;36:472-4.
3. Conte, JV, Arcomano, TA, Naficy, MA, Holt, RW. Treatment of bleeding stomal varices. Report of a case and review of the literature. Dis Colon Rectum 1990;33:308-14.

Figure 3. *Computer tomography of the abdomen of the patient with oral contrast showing large aberrant vascular structures running to the stoma*



Aims and scope
The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

Manuscripts
Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Language
The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

Submission
All submissions to the *Netherlands Journal of Medicine* should be submitted online through Manuscript Central at http://mc.manuscriptcentral.com/nethjmed. Authors should create an account and follow the instructions. If you are unable to submit through Manuscript Central contact the editorial office at g.derksen@aig.umcn.nl, tel.: +31 (0)24-361 04 59 or fax: +31 (0) 24-354 17 34.

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process, number the lines in the margin and the pages.
Subheadings should not exceed 55 characters, including spaces.
Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for generic names of substances and materials.
A *Covering letter* should accompany the manuscript, identifying the corresponding person (with the address, telephone number, fax number and e-mail address). Conflicts of interest, commercial affiliations, consultations, stock or equity interests should be specified. In the letter one to three sentences should be dedicated to what this study adds. The letter should make it clear that the final manuscript has been seen and approved by all authors. All authors should sign the letter. The letter should either be submitted through http://mc.manuscriptcentral.com/nethjmed or faxed to the editorial office (+31 (0)24-354 17 34).

Divide the manuscript into the following sections: Title page, Abstract, Keywords, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

The *Title page* should include authors' names, degrees, academic addresses, correspondence address, including telephone number, fax number, e-mail address and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

The *Abstract*, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: Background, Methods, Results and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

Keywords: Include three to five keywords.

The *Introduction* should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely, without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

Acknowledgement: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.

References should be numbered consecutively as they appear in the text (after the punctuation and in square brackets). Type the reference list with double spacing on a separate page. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med 1991;324:424-8).

Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.
2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

Please note that all authors should be listed when six or less; when seven or more, list only the first three and add et al. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against the reference list after your manuscript has been revised.
The use of bibliographic software programmes that are designed to generate reference lists such as Reference Manager© or Endnote© is highly encouraged. Authors can use the predefined output 'Vancouver' style from these programmes.

Tables should be typed with double spacing each on a separate page, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors.
Legends for figures should be typed, with double spacing, on a separate page.

Case reports
Case reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in the Netherlands Journal of Medicine. Neth J Med 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references.

Mini reviews
Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

Letters to the editor (correspondence)
Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please use SI units for measurements and provide the references conform the Vancouver style (N Engl J Med 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the Journal, the referred article should be quoted in the list of references.

Photo quiz
A photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

Book reviews
The editorial board will consider articles reviewing books.

Reviewing process
After external and editorial review of the manuscript the authors will be informed about acceptance, rejection or revision. We require revision as stated in our letter.

Proofs
Proofs will be sent to the authors to be carefully checked for printer's errors. Changes or additions to the edited manuscript cannot be allowed at this stage. Corrected proofs should be returned to the editorial office within two days of receipt.

Offprints
These are not available. The first author receives a sample copy of the Journal with the published article.