Diabetologia (1993) 36: 826–828

Diabetologia

Reversible impairment of glucose tolerance in patients with endemic fluorosis

N. Trivedi, A. Mithal, S. K. Gupta, M. M. Godbole for the Fluoride Collaborative Study Group

Department of Medical Endocrinology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, India

Summary. Endemic fluorosis is a condition resulting from prolonged ingestion of drinking water which contains excess fluoride. Studies on rats have suggested that fluoride toxicity may produce glucose intolerance and abnormalities in insulin secretion. We studied glucose and insulin profiles following an oral glucose load in patients with endemic fluorosis. Twenty-five young adults (age range, 15–30 years) with endemic fluorosis, and an equal number of matched healthy control subjects with normal fluoride intake were studied. Impaired glucose tolerance was demonstrated in 10 of 25 (40%) patients with endemic fluorosis. Patients with impaired glucose tolerance had significantly higher fasting serum immunoreactive insulin ($p < 0.05$), higher fasting serum fluoride ($p < 0.001$), and a significantly lower fasting glucose to insulin ratio than that in patients with normal glucose tolerance ($p < 0.001$) or control subjects ($p < 0.05$). The fasting serum fluoride levels correlated positively with the area under the glucose curve ($r = 0.80$, $p < 0.01$) in patients with impaired glucose tolerance. Interestingly these abnormalities could be reversed when the village was provided drinking water with fluoride levels within acceptable limits. The present study shows that chronic fluoride toxicity in humans could result in significant abnormalities in glucose tolerance which are reversible upon removal of the excess fluoride.

Key words: Fluoride, glucose, insulin, insulin resistance.

Endemic fluorosis, a disorder mainly affecting dental and skeletal tissues, is a major public health problem in many countries in Africa and Asia including India [1, 2]. Although an increased fluoride load has been shown to induce changes in insulin and glucose levels in previously normal experimental animals, [3, 4] this has not been studied in patients with endemic fluorosis. We therefore studied the glucose and insulin profile after an oral glucose load in patients with skeletal fluorosis residing in a highly endemic area, in the state of Uttar Pradesh in Northern India.

Subjects and methods

Subjects

We studied 25 patients (14 males, 11 females; age range 15 to 30 years) who had clear evidence of skeletal and dental fluorosis on clinical and radiological evaluation. All the subjects had been consuming water with fluoride levels above the acceptable limit [5] since birth (i. e. water from shallow wells, fluoride levels ranging from 2 to 13 mg/l), and were excreting high levels of fluoride in their urine (range 2–8 mg/l). The subjects were non-obese, non-smokers, postpubertal, and were not taking any medication. They had no history of overt diabetes mellitus or hypertension or family history of these diseases. The control group consisted of 25 age-, sex- and BMI-matched normal individuals with comparable social and working conditions, except that they were consuming water with acceptable fluoride content (< 1 mg/l), and their urine fluoride levels ranged from 0.2 to 0.5 mg/l.

Methods

The patients and control subjects were consuming their normal diets at the time of study. After an overnight fast they drank a 75-g glucose solution. Venous blood was obtained in the fasting state and at 60 and 120 min after the oral glucose load. Sera were stored at −20°C.

Samples were assayed for glucose (glucose oxidase method) and immunoreactive insulin (IRI, double antibody separation, intra- and inter-assay variation < 10%). Fluoride levels (ion-specific electrode, F9409; Orion Research, Boston, Mass., USA) were also estimated in the fasting serum sample. Fasting glucose: insulin (G:I) ratio was calculated by dividing serum glucose in mmol/l by serum immunoreactive insulin in μU/ml; and area under glucose and insulin curves was calculated by the trapezoid rule.

The village was provided with an alternative water source within a month (i. e. water from deep wells, with fluoride content < 1 mg/l).

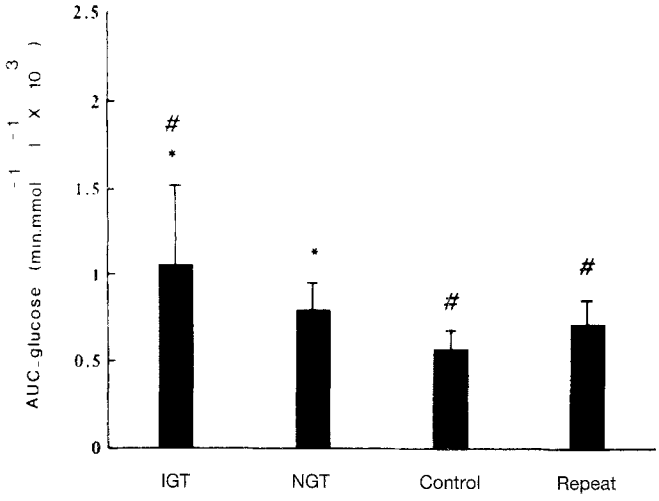

Fig. 1. Area under glucose curve (AUC-glucose) in endemic fluorosis patients with impaired glucose tolerance (IGT, $n = 10$); endemic fluorosis patients with normal glucose tolerance (NGT, $n = 15$); control subjects (Control, $n = 25$), and IGT patients after 6 months of normal fluoride intake (Repeat, $n = 10$). * $p < 0.01$, # $p < 0.001$ IGT vs other groups

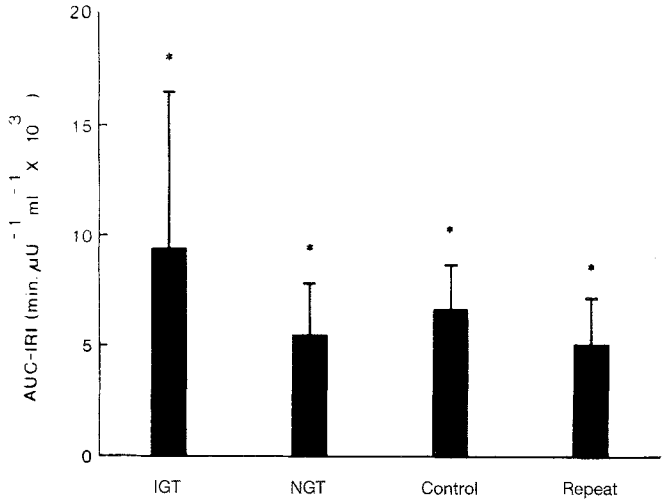

Fig. 2. Area under insulin curve (AUC-IRI) in endemic fluorosis patients with impaired glucose tolerance (IGT, $n = 10$); endemic fluorosis patients with normal glucose tolerance (NGT, $n = 15$); control subjects (Control, $n = 25$), and IGT patients after 6 months of normal fluoride intake (Repeat, $n = 10$). * $p < 0.05$ IGT vs other groups

Table 1. Glucose and insulin profile in patients and control subjects

Parameter	IGT ($n = 10$)	NGT ($n = 15$)	Control subjects ($n = 25$)	Repeat ($n = 10$)
Glucose (mmol/l)				
fasting	4.8 (1.1)[a]	5.8 (1.1)[a]	4.3 (0.8)	4.6 (0.8)
60 min	10.0 (4.5)[a, b, c]	7.7 (1.5)[a]	5.5 (1.6)[c]	7.0 (1.8)[b]
120 min[d]	10.6 (5.3)[c]	5.7 (1.2)[c]	3.9 (1.4)[c]	5.5 (1.3)[c]
IRI (μU/ml)				
fasting	22.2 (23.6)[a]	9.1 (8.4)[a]	9.4 (3.4)[a]	8.9 (4.8)[a]
60 min	82.4 (61.4)	65.6 (47.8)	51.9 (33.9)	82.5 (32.5)
120 min	61.2 (55.8)	42.8 (21.2)	63.8 (38.5)	52.0 (40.2)
Fasting glucose: insulin ratio	0.36 (0.31)[a,c]	0.87 (0.43)[c]	0.55 (0.17)[a]	0.62 (0.33)[a]

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ IGT patients vs other groups. Values are given as mean (SD).
IGT, Endemic fluorosis patients with impaired glucose tolerance;
NGT, endemic fluorosis patients with normal glucose tolerance;
Control subjects, healthy individuals with normal fluoride intake;
Repeat, repeat oral glucose test in IGT patients;
IRI, serum immunoreactive insulin.
[d] one patient had a glucose value of 17.3 mmol/l in IGT group

The tests were repeated in those subjects with impaired glucose tolerance (IGT, $n = 10$) using a similar protocol after a period of 6 months. During these 6 months the subjects continued to consume their normal diet.

Statistical analysis

Statistical analysis was performed using Student's *t*-test, two-way analysis of variance (ANOVA) and correlation coefficient wherever applicable. *p* values less than 0.05 were considered to be significant.

Results

Of 25 patients with endemic fluorosis, 10 (6 males, 4 females) had IGT according to World Health Organisation criteria. Endemic fluorosis patients with IGT had significantly higher ($p < 0.001$, two-way ANOVA) serum glucose levels for repeated measurements after glucose load; they also had higher area under glucose curve (Fig. 1), fasting serum immunoreactive insulin (Table 1) and area under insulin curve (Fig. 2) than those patients with normal glucose tolerance (NGT) and healthy control subjects. The fasting serum G:I ratio was significantly lower in patients with IGT than those with NGT and healthy control subjects (Table 1). Serum fluoride levels were significantly higher in patients with IGT (0.08 ± 0.04 mg/l) than those with NGT (0.02 ± 0.01 mg/l, $p < 0.001$) and healthy control subjects (0.01 ± 0.009 mg/l, $p < 0.001$).

The serum fluoride levels correlated positively with area under glucose curve ($r = 0.80$, $p < 0.01$) in patients with IGT, but fasting IRI, area under insulin curve and fasting G:I ratio showed no correlation with serum fluoride in these patients. However, serum fluoride showed a significant positive correlation with fasting IRI ($r = 0.54$, $p < 0.05$), and a significant negative correlation with fasting G:I ratio ($r = -0.49$, $p < 0.01$) when all 25 patients with endemic fluorosis were analysed together. Serum calcium, inorganic phosphorus and 25 hydroxy-vitamin D levels were normal, and serum alkaline phosphatase higher in patients with endemic fluorosis (data not shown).

Effect of provision of safe water

After 6 months of normal fluoride intake IGT normalised in the patients with endemic fluorosis, the fasting serum IRI decreased and fasting G:I ratio increased significant-

ly (Table 1). The serum fluoride levels (0.02 ± 0.01 mg/l) also fell significantly as compared to initial levels in patients with IGT ($p < 0.001$).

Discussion

Our study has shown for the first time that 40% of young patients with endemic fluorosis have IGT, with elevated serum IRI and low fasting G : I ratios. The serum fluoride levels correlated positively with area under glucose curve in these patients.

The mechanism of fluoride-induced glucose intolerance and alteration in insulin levels is not clear. Glycosuria [6] and hyperglycaemia [7, 8] have been demonstrated in experimental rats exposed to fluoride, and may be due to an increase in hepatic glucose 6-phosphatase activity [7, 8]. After acute administration of fluoride in rats insulin secretion is inhibited, which led to hyperglycaemia [3]. In contrast Howell and Montague [9] have demonstrated that fluoride enhances production of cAMP in isolated islets of Langerhans from rats, resulting in increased insulin secretion. Chronic fluoride toxicity in intact experimental rats has been shown to produce subtle abnormalities in glucose tolerance without significantly altering insulin levels [4].

Our study showed that fasting serum IRI was significantly higher in patients with IGT. Elevated serum IRI along with elevated serum glucose indicate either secretion of bio-inactive insulin or insulin resistance. In the absence of a specific assay for proinsulin, elevated IRI in our study could reflect elevated proinsulin which has lower bioactivity. Insulin resistance, a potentially deleterious condition [10] could also be occurring in our study subjects and warrants further investigation.

The present study shows that a substantial proportion of young adults with endemic fluorosis have IGT, which is reversible upon removal of the fluoride load. This finding could have major implications for countries where millions are affected by fluorosis [1] and perhaps also for fluoride-treated osteoporotic subjects. Thus further studies are warranted.

Acknowledgements. We are extremely grateful to Professor K. Kojima (Team Leader, Japan International Cooperation Agency, SGPGIMS, Lucknow), Dr. C. M. Pandey (Department of Biostatistics, SGPGIMS, Lucknow) and Dr. N. Satsangi (Pool officer, Department of Endocrinology, SGPGIMS, Lucknow) for their help.

References

1. Mangla B (1991) India: defluoridation battle. Lancet 337: 1213
2. World Health Organisation (1984) Enviroment Health Criteria-36. Fluorine and Fluoride. World Health Organisation, Geneva
3. Rigalli A, Ballina JC, Roveri E, Puche RC (1990) Inhibitory effect of fluoride on insulin secretion. Calcif Tissue Int 46: 333–338
4. Rigalli A, Ballina JC, Puche RC (1992) Bone mass increase and glucose tolerance in rats chronically treated with sodium fluoride. Bone Mineral 16: 101–108
5. World Health Organisation (1984) Guidelines for drinking water quality. Volume 1, recommendations. World Health Organisation, Geneva
6. Taylor JM, Scott JK, Maynard EA, Smith FA, Hodge HC (1961) Toxic effects of fluoride on rat kidneys. I. Acute injury from large single doses. Toxicol Appl Pharmacol 3: 278–289
7. Suketa Y, Asao Y, Kanamoto Y, Shakashita T, Okada S (1985) Changes in adrenal function as a possible mechanism for elevation of serum glucose by single large dose of fluoride. Toxicol Appl Pharmacol 80: 199–205
8. Suketa Y, Sato M (1980) Changes in glucose-6-phosphatase activity in liver and kidneys of rats treated with single large dose of fluoride. Toxicol Appl Pharmacol 52: 386–390
9. Howell SL, Montague W (1973) Adenylate cyclase activity in isolated rat islet of Langerhans: effect of agents which alter insulin secretion. Biophys Biochem Acta 320: 44–52
10. Caro JF (1991) Insulin resistance in obese and nonobese man. J Clin Endocrinol Metab 73: 691–695

Received: 26 February 1993
and in revised form: 7 April 1993

Dr. A. Mithal
Department of Medical Endocrinology
Sanjay Gandhi Post-Graduate
Institute of Medical Sciences
P.O. Box 375
Lucknow 226001
India