CLIN. CHEM. 34/12, 2418–2422 (1988)

Increased Urinary Excretion of Digoxin-like Immunoreactive Substance by Insulin-Dependent Diabetic Patients: a Linkage with Hypertension?

Ottavio Giampietro, Aldo Clerico, Giovanna Gregori, Stefania Bertoli, Maria Grazia Del Chicca, Roberto Miccoli, Amalia Lucchetti, Lorella Cruschelli, and Renzo Navalesi

Excretion of digoxin-like immunoreactivity (DLIS) was measured by RIA in timed overnight urine collections from 91 normotensive nondiabetic subjects and 104 normotensive insulin-dependent diabetic (IDDM) patients. The mean ± SD DLIS excretion rate for the diabetic patients significantly exceeded that for the controls (73 ± 41 vs 63 ± 36 pg/min, $P = 0.024$). In both groups, the mean DLIS excretion rates for men were significantly higher ($P = 0.0014$, $P = 0.0006$) than for women. In the controls, the DLIS excretion rate significantly correlated with the urinary excretion rate of creatinine ($P < 0.01$), Na^+ ($P < 0.05$), and K^+ ($P < 0.05$), and with the subjects' body weight ($P < 0.01$), body mass index ($P < 0.05$), and systolic blood pressure ($P < 0.05$). In the diabetics, the DLIS excretion rate was significantly correlated with body weight ($P < 0.05$) and with urinary excretion rates for albumin ($P < 0.01$), creatinine ($P < 0.01$), Na^+ ($P < 0.05$), and K^+ ($P < 0.05$). Our data indicate that: (*a*) increased amounts of a cardiac glycoside-like substance (or a group of substances) are excreted in the urine of IDDM patients; (*b*) the urinary excretion of DLIS seems to depend on glomerular filtration rate and physicochemical properties of glomerular membrane, as well as on subjects' body mass; and (*c*) because cardiac glycoside-like substances may increase peripheral vascular resistance, increased urinary excretion of DLIS by IDDM patients may indicate a tendency to develop hypertension.

Additional Keyphrases: *albuminuria · sodium · potassium · creatinine · nephropathy · sex-related difference*

Considerable evidence (*1, 2*) indicates that increases in body sodium and expansion of extracellular volume lead to the release of humoral natriuretic factors. As recently reviewed (*1–4*), one of these factors is suggested to be an endogenous substance having immunological and biological activities similar to those of the cardiac glycosides. This endogenous factor inhibits Na^+/K^+-transporting ATPase (EC 3.6.1.37) in various tissues, including the kidney, and this causes natriuresis.

Insulin-dependent diabetes mellitus (IDDM) reportedly is associated with sodium retention, leading to increases of exchangeable body sodium (*5, 6*) and extracellular volume and a suppression of the renin–angiotensin–aldosterone system.[1] This has been recently confirmed by Feldt-Rasmussen et al. (*7*). Thus, the release of a substance with cardiac glycoside-like activity and Na^+/K^+-ATPase inhibiting activity could conceivably be triggered by volume expansion in IDDM. In fact, inhibition of Na^+/K^+-ATPase has been documented in tissues of experimental diabetic animals and in diabetic patients (*8*).

Several studies (for review, see references *1–4*) indicate that, in addition to its natriuretic activity, this cardiac glycoside-like endogenous factor, by inhibiting Na^+/K^+-ATPase in arterioles, increases the intracellular Na^+, which in turn increases the intracellular calcium, leading to increased vascular reactivity. Hence, endogenous factors with activity similar to that of cardiac glycosides may play a role in the pathogenesis of some forms of hypertension, especially the low-renin forms (*1–4*). Such could also be the case in IDDM.

Hypertension and diabetes mellitus may occur together (*9, 10*), and hypertension is more prevalent in diabetic patients than in the general population (*10, 11*). Diabetic nephropathy is considered the main cause of hypertension in IDDM (*12*), and blood pressure may begin to increase even in patients with slight albuminuria, who are at high risk of developing overt diabetic nephropathy (*13*). However, the hypertension–diabetes association in IDDM patients cannot be entirely secondary to diabetic nephropathy, because hypertension can already be present in patients with recent onset of diabetes (*14*) but without signs of renal involvement (*10, 11*). Because of abnormalities in the balance of body sodium and fluid volume in IDDM patients (*5–7*), the release of an endogenous factor with cardiac glycoside-like activity could play a role in the pathogenesis of non-nephropathic hypertension in IDDM.

To test the hypothesis that production of a substance with cardiac glycoside-like activity might be increased in IDDM uncomplicated by overt diabetic nephropathy or hypertension, we used a sensitive RIA method (*15, 16*) to measure the urinary excretion rate of a substance with digoxin-like immunoreactivity (DLIS) in 104 normotensive, non-nephropathic IDDM patients and 91 nondiabetic control subjects. In each of these groups (controls and diabetics), we also measured the urinary albumin excretion rate, an early and sensitive predictor of clinical diabetic nephropathy (*13*), and some other clinical, metabolic, and renal characteristics of the subjects, to determine their relationship, if any, with urinary DLIS excretion rate.

Materials and Methods

Subjects. Ninety-one nondiabetic, normotensive subjects (46 men, 45 women) and 104 normotensive, Albustix-negative (Miles GmbH, Sparte Ames, Frankfurt, F.R.G.) IDDM patients (41 men, 63 women) participated in our study. No subject was being treated with digoxin. All subjects had a normal renal function, as assessed by endogenous creatinine clearance, and all, except for 10 diabetics, were normoalbuminuric. Some other pertinent clinical characteristics are reported in Table 1. For the slight ("pauci") albuminuria threshold, we chose the albumin excretion rate of 30 μg/min, in accord with Viberti et al. (*13*). We verified by culturing that all subjects' urines were sterile. Of the diabetics, 62

Cattedra di Malattie del Ricambio, Istituto di Clinica Medica II, C.N.R. Institute of Clinical Physiology, University of Pisa, Via Roma 67, 56100 Pisa, Italy.

[1] Nonstandard abbreviations: IDDM, insulin-dependent diabetes mellitus; DLIS, digoxin-like immunoreactive substance; HbA_{1c}, glycated hemoglobin.

Received June 14, 1988; accepted September 2, 1988.

Table 1. Clinical Characteristics of Subjects Studied (Means ± SD)

	Control subjects	IDDM	
		AER <30 μg/min	AER >30 μg/min
n	91	94	10
Age, y	30.2 ± 10.1	31.7 ± 13.2	37.6 ± 14.6
BMI, kg/m²[a]	22.8 ± 3.0	23.1 ± 3.8	24.8 ± 3.6
Diabetes duration, y	—	9.6 ± 7.1	16.0 ± 4.5
Insulin, units/day	—	53.4 ± 21.2	58.2 ± 19.4
PPG, mg/L	785 ± 118	1589 ± 697[b]	1713 ± 619[b]
HbA_{1c}, %	5.3 ± 0.4	8.4 ± 1.8[b]	10.0 ± 1.6[b]
AER, μg/min	4.6 ± 2.7	6.9 ± 5.4[b]	66.5 ± 39.6[b,c]
Serum Cr, mg/L	8.0 ± 1.0	8.0 ± 2.0	8.0 ± 4.0
Blood pressure, mmHg			
Systolic	116.2 ± 9.6	120.0 ± 15.0	122.1 ± 15.0
Diastolic	76.9 ± 6.4	75.6 ± 11.8	77.9 ± 7.0

[a] Body mass index: body weight/height². [b] Significantly different ($P < 0.01$) from control subjects. [c] Significantly different ($P < 0.01$) from IDDM subjects with AER <30 μg/min. Abbreviations: AER, albumin excretion rate; PPG, preprandial plasma glucose; Cr, creatinine.

(59.6%) had no signs of retinopathy, 29 (27.9%) had background retinopathy, and 13 (12.5%) had retinal proliferative lesions. All patients were treated with insulin therapy only, most of them with multiple insulin injections (the mean daily doses are listed in Table 1).

Every subject collected urine specimens before going to bed and upon arising, and the time of collection was exactly recorded for the calculation of urinary DLIS excretion rate (in pg/min) and albumin excretion rate (in μg/min). For most of these subjects we also calculated the urinary excretion rate for creatinine (mg/min) and electrolytes (Na^+ and K^+, mmol/min). When the same subjects collected more than one urine specimen on consecutive nights (as did 26 of 104 diabetics and 43 of 91 control subjects), we used for statistical calculations the mean rate of urinary excretion of each analyte from the several collections.

DLIS assay. We measured DLIS by a solid-phase RIA (*15, 16*) in which digoxin dissolved in a buffer containing human serum albumin, 40 g/L, is used as standard, ^{125}I-labeled digoxin is the tracer, and antibody-coated test tubes are used to separate bound from free digoxin (tracer and coated test tubes were supplied by Byk Gulden Italia S.p.A., Cormano, Italy). To improve the sensitivity and the reproducibility of the assay as well as the stability of standard curves, we also included human serum albumin, 40 g/L, in the assay buffer and added the same albumin-containing buffer to unknown urine samples, to equalize the effect of albumin on the reaction volume. Results were expressed as digoxin equivalents. We assayed 0.2-mL urine samples in duplicate, without dilution or extraction. The mean limit of detection (95% confidence limit), as determined in 21 separate experiments, was 5.38 (SD 1.07) pg per tube. The mean between-assay CV for DLIS in a urine pool, assayed over 10 months (n = 25), was 12.9% (mean DLIS concn, 281.9, SD 36.4 ng/L).

Albumin excretion rate assay. We used an RIA kit ("Albumin RIA 100"; Pharmacia AB, Uppsala, Sweden) for assay of albuminuria in normal subjects and diabetics. This RIA involves purified human serum albumin as the standard, ^{125}I-labeled human serum albumin as tracer, and a specific antiserum to human serum albumin. Bound and free phases were separated by a solid-phase system (sheep anti-rabbit IgG, bound to Sepharose). We assayed, in duplicate, 50-μL urine samples; the standard curve was based on assays of solutions containing known amounts of purified human serum albumin (0.8, 1.6, 4, 8, 16, 40, and 80 mg/L). The RIA was not subject to interference from bovine serum albumin, transferrin, or human immunoglobulins. The mean (n = 25) limit of detection of this RIA was 0.28 (SD 0.13) mg/L. The between-assay CV for a wide range of albumin concentrations in several pooled-urine specimens ranged between 5% and 15%.

Other methods. We measured glucose in plasma by a glucose oxidase (EC 1.1.3.4) method, in a glucose analyzer (Beckman Instruments, Fullerton, CA). To quantify glycated hemoglobin (HbA_{1c}), we used an automated HPLC analyzer system (HA-8110; Daiichi Kagaku, Kyoto, Japan). Creatinine in serum and urine was measured by the Jaffé reaction (Astra 8, Beckman), urinary sodium and potassium by flame photometry (Model 435 flame photometer; Corning Medical, Healsted, Essex, U.K.).

Statistical analysis. All values for samples and the other data for quality control of the two RIA systems were calculated by use of previously described computer programs (*17, 18*).

The interpolation of the dose–response curves was calculated by using a four-parameter logistic function (*18*).

For statistical analysis for comparison of groups by parametric and nonparametric tests and calculation of simple and multiple regression coefficients we used a Mackintosh SE personal computer with the "512 + Stat-View" and "Stat-View" programs. All data are reported as mean ± SD.

Results

Figure 1 shows the frequency distribution of values for urinary DLIS excretion observed in the diabetic patients and matched controls. The values for either group were not normally distributed.

The mean DLIS excretion rate for men significantly exceeded that for women, both in control subjects and diabetics (control subjects: women 51.6 ± 21.4 pg/min vs men 73.7 ± 42.7 pg/min, $P = 0.0006$ by the Mann–Whitney U two-tailed test; diabetics: women 65.6 ± 39.2 pg/min vs men 86.7 ± 41.4 pg/min, $P = 0.0014$).

Diabetic patients (men and women) had a mean DLIS excretion rate significantly higher than controls (73.09 ± 41.21 pg/min vs 62.80 ± 35.49 pg/min, $P = 0.024$ by the Mann–Whitney U two-tailed test). Even taking into account the sex of subjects, diabetics had a mean DLIS excretion rate significantly higher than controls (diabetic vs control men: $P = 0.038$; diabetic vs control women: $P = 0.0316$, Mann–Whitney U two-tailed test) (Figure 2).

After the 10 paucialbuminuric patients (albumin excretion rate >30 μg/min) were excluded from statistical analy-

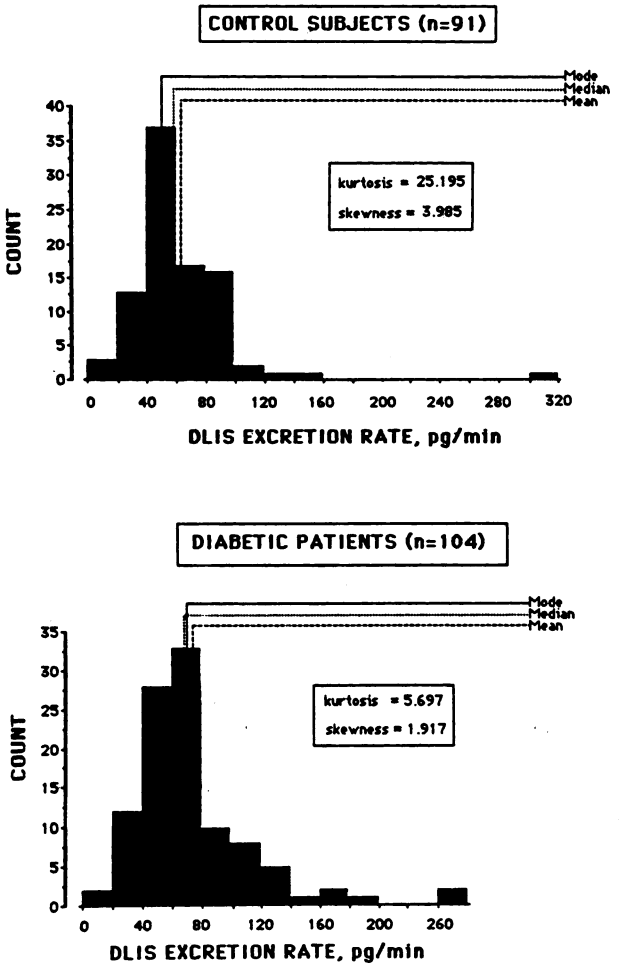

Fig. 1. Frequency distribution of values for urinary DLIS excretion rates in control subjects and IDDM patients

sis, diabetics as a group showed still a significantly higher DLIS excretion rate (71.036 ± 37.449, n = 94, by Mann–Whitney one-tailed test $P = 0.032$) than control subjects. The 10 paucialbuminuric patients showed a DLIS excretion rate (100.88 ± 63.63 pg/min) significantly higher than the 94 normoalbuminuric diabetics ($P < 0.05$) and the control subjects ($P < 0.006$) by the Mann–Whitney one-tailed test. Therefore, the DLIS excretion rate values of three groups (controls, normoalbuminuric, and paucialbuminuric diabetics) do not come from the same population (Kruskal–Wallis one-way analysis, $P < 0.02$). The paucialbuminuric patients who, all except one, had retinopathy (three background, six proliferative), also showed significantly higher values for duration of diabetes (16 ± 4 years, by t-test $P < 0.01$) and HbA_{1c} (10% ± 2%, by t-test $P < 0.02$) than did normoalbuminuric diabetics. Finally, blood pressure tended to be higher (t-test, $P > 0.05$) in paucialbuminuric (systolic 122.1 ± 14.7 mmHg; diastolic, 77.9 ± 7.0 mmHg) than in normoalbuminuric diabetic patients [119.8 (SD 14.7) and 75.6 (SD 11.8) mmHg, respectively] (Table 1).

In the control group, urinary DLIS excretion rate significantly correlated with urinary creatinine, Na^+, and K^+ excretion rates, as well as with subjects' body weight, body mass index, and systolic blood pressure, but not with their age, albumin excretion rate, serum creatinine, and diastolic blood pressure (Table 2). In the stepwise regression analysis, only urinary creatinine excretion rate significantly contributed to regression.

In diabetic patients, the DLIS excretion rate significantly correlated with the excretion rates for urinary albumin, creatinine, Na^+, and K^+, and also with the body weight of patients, but not with their age, blood pressure, diabetes duration, HbA_{1c}, serum creatinine, or presence and severity of retinopathy (Table 2). In the multiple regression analysis, not only urinary creatinine excretion rate (the first variable entered), but also albumin excretion rate significantly contributed to the correlation with DLIS excretion rate in diabetic patients ($r = 0.517$, $P < 0.001$, n = 63). The correlations between DLIS excretion rate and albumin excretion rate, and DLIS excretion rate and creatinine excretion rate, respectively, were still present also after the 10 paucialbuminuric patients had been excluded from the regression.

Discussion

Because DLIS concentrations in plasma of normal adults and hypertensive patients are often near the detection limit of our RIA method (about 25 pg/mL, digoxin equivalents), direct assay of plasma samples (i.e., without extraction and concentration of samples) is not indicated (*19*). On the contrary, because urine has three- to 10-fold higher concentrations of DLIS than those in blood, assay of urine samples seems preferable for clinical studies (*19*). The present study is the first one evaluating the urinary excretion of DLIS in a large number of normal subjects and IDDM patients. The timed overnight urine collection was chosen because it is easier to perform than 24-h collection, and because physical activity affects values for DLIS in both serum and urine (*20, 21*).

We measured DLIS values by a very sensitive RIA method specifically modified in our laboratory for the assay of digoxin-like immunoreactivity (*16, 19*). As previously reported for extracts of plasma of adults, pregnant women, and newborns, the results obtained with this RIA correlate well with those by a radioreceptor assay based on binding of [^{3}H]ouabain to human erythrocytes (*22*). So this RIA can

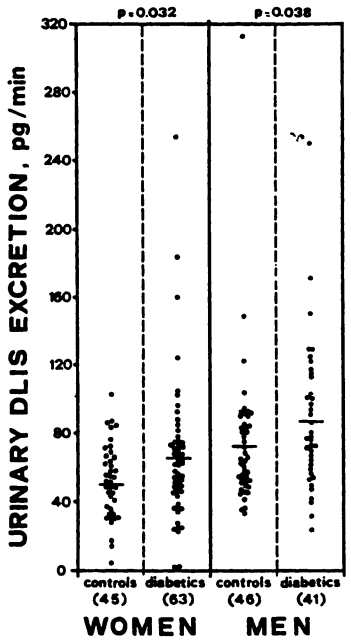

Fig. 2. Values for DLIS excretion rate, by sex, in controls and IDDM patients

Horizontal bars indicate the means for each group. Significance of the differences is indicated at top

detect, to some extent, substances with both immunological and biological activity similar to that of cardiac glycosides.

Insulin-dependent diabetic patients, normotensive and with normal renal function, were chosen for the study because DLIS can be increased in biological fluids of patients with essential hypertension and with renal function impairment (*3, 4*).

In the present study, the urinary DLIS excretion rate was significantly smaller in women than in men, both in diabetics and controls, and was significantly related to subjects' body weight and body mass index. Evidently, DLIS urinary output depends closely on subjects' body mass and frame.

Urinary DLIS excretion rate was significantly higher in IDDM than in nondiabetic subjects. The overlap between the two groups (Figures 1 and 2) may be because we studied only normotensive diabetic patients, most of whom were in fair or good metabolic control and none of whom had clinical diabetic nephropathy. Preliminary findings from the 10 paucialbuminuric patients seem to suggest that IDDM patients with overt or incipient (*23*) nephropathy and (or) in poor metabolic control could have greater DLIS excretion in urine. The 10 paucialbuminuric diabetics tend also to have marginally increased blood pressure, although still within the normal range, in accord with previous reports (*23*). This marginally increased blood pressure conceivably could play a role in determining the increased urinary DLIS excretion.

Owing to its significant correlation with creatinine excretion rate in controls, and with creatinine excretion rate and albumin excretion rate in diabetic patients, the urinary excretion of DLIS seems conceivably to depend both on glomerular filtration and on physicochemical properties of glomerular membrane.

Among mechanisms that may contribute to the abnormality in the balance of body sodium and fluid volume reported in IDDM patients (*5–7*), peripheral hyperinsulinemia caused by exogenous insulin administration may be included (*7*). The hyperinsulinemia stimulates reabsorption of sodium by the kidney (*7, 23–25*), ultimately leading to an increase in exchangeable sodium and to an expanded extracellular volume (*5–7, 25*). In addition, diabetic patients, mainly insulin-dependent ones, tend to have high circulating concentrations of somatotropin (*26*), which itself stimulates tubular reabsorption of sodium (*27, 28*). Continued expansion of the body extracellular volume by sodium retention can thus stimulate the release of a circulating digitalis-like substance in IDDM patients (*1–4*). The significant correlation we found between urinary excretion rates of DLIS and Na^+ is

Table 2. Coefficients of Correlation between Urinary DLIS Excretion Rate and Other Clinical Characteristics in Control Subjects and Diabetic Patients

	Controls	Diabetics
	r	
Age	0.019	0.111
Body weight	0.335[a]	0.258[b]
Body mass index	0.250[b]	0.254[b]
Systolic blood pressure	0.285[b]	0.033
Diastolic blood pressure	0.066	0.020
Diabetes duration	—	0.098
HbA_{1c}	—	0.045
Albumin excretion rate	0.026	0.264[a]
Na^+ excretion rate	0.283[b]	0.281[b]
K^+ excretion rate	0.320[b]	0.298[b]
Creatinine excretion rate	0.567[a]	0.398[a]

Correlations are significant at [a] $P < 0.01$, [b] $P < 0.05$.

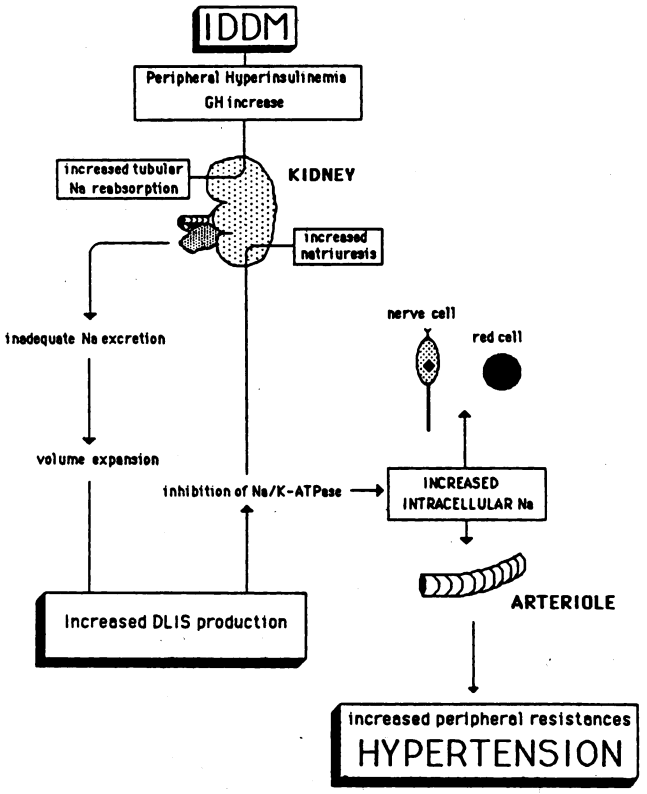

Fig. 3. Working hypothesis for the pathogenesis of hypertension in IDDM

in harmony with this hypothesis. In addition, Na^+/K^+-ATPase activity has been found to be decreased in certain tissues of experimental diabetic animals—peripheral nerves, sarcolemma, blood–brain barrier (*8, 29, 30, 31*)—and in erythrocytes of type 1 diabetic patients (*32*). These findings suggest that, in IDDM, the tissues are probably in contact with increased amounts of an endogenous inhibitor of Na^+/K^+-ATPase.

Diabetes mellitus and hypertension are chronic conditions that frequently coexist (*9*). The increased urinary excretion rate of digoxin-like immunoreactivity in a group of normotensive, non-nephropathic IDDM patients could lead us to consider the increased urinary DLIS as a link with hypertension in IDDM.

As a working hypothesis (Figure 3), the release of an endogenous natriuretic factor with biological and immunological cardiac properties could cause a stable hypertension in genetically susceptible IDDM patients (*33–35*).

Further longitudinal studies are needed to definitively disclose and assess the pathophysiological and clinical relevance of increased urinary DLIS excretion in IDDM patients.

This work was performed in the context of the Ricerca Finalizzata della Regione Toscana. In addition, it was supported in part by a grant (84.02442.56) from the National Research Council and by a grant from the Ministero della Pubblica Istruzione (Ricerca Scientifica 1986).

References

1. De Wardener HE, Clarkson EM. Concept of natriuretic hormone [Review]. Physiol Rev 1985;65:658–759.

2. Buckalew Jr VM, Gruber KA. Natriuretic hormone [Review]. Annu Rev Physiol 1984;46:343–58.

3. Graves SW, Williams GH. Endogenous digitalis-like natriuretic factors [Review]. Annu Rev Med 1987;38:433–4.

4. Clerico A. Digoxin-like immunoreactivity, endogenous cardiac glycoside-like factor(s) and natriuretic hormone: more than an

hypothesis [Review]. J Nucl Med Allied Sci 1987;31:341–56.

5. Weidmann P, Beretta-Piccoli C, Trost BN. Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. Hypertension 1985;7(Suppl 2):33–42.

6. O'Hare JA, Ferriss JB, Brady D, Twomey B, O'Sullivan DJ. Exchangeable sodium and renin in hypertensive diabetic patients with and without nephropathy. Hypertension 1985;7(Suppl 2):43–8.

7. Feldt-Rasmussen B, Mathiesen ER, Deckert T, et al. Central role for sodium in the pathogenesis of blood pressure changes independent of angiotensin, aldosterone and catecholamines in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1987;30:610–7.

8. Green DA, Lattimer SA, Sima AAF. Sorbitol, phosphoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications. N Engl J Med 1987;316:599–606.

9. Weidmann P, Mogensen CE, Ritz E. Hypertension associated with diabetes mellitus. Hypertension 1985;7(Suppl 2):1–2.

10. The Working Group on Hypertension in Diabetes. Statement on hypertension in diabetes mellitus. Final report. Arch Intern Med 1987;147:830–42.

11. Christlieb AR, Krolewski AS, Warram JH, Soeldner JS. Is insulin the link between hypertension and obesity? Hypertension 1985;7(Suppl 2):54–7.

12. Pyorala K, Uusitupa M, Laakso M. Hypertension and diabetes: a poorly understood relationship. In: Andreani D, Crepaldi G, Di Mario U, Pozza G, eds. Diabetic complications: early diagnosis and treatment. New York: John Wiley & Sons, 1987:43–51.

13. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982;i:1430–2.

14. Pell S, D'Alonzo CA. Some aspects of hypertension in diabetes mellitus. J Am Med Assoc 1967;202:104–10.

15. Balzan S, Clerico A, Del Chicca MG, Montali U, Ghione S. Digoxin-like immunoreactivity in normal human plasma and urine, as detected by a solid-phase radioimmunoassay. Clin Chem 1984;30:450–1.

16. Clerico A, Del Chicca MG, Montereggi A, Balzan S, Ghione S. Digoxin-like immunoreactivity in human body fluids. J Nucl Med Allied Sci 1985;29:305–12.

17. Pilo A, Zucchelli GC. Automatic treatment of radioimmunoassay data: an experimental validation of the results. Clin Chim Acta 1975;64:1–4.

18. Pilo A, Zucchelli GC, Malvano R, Masini S. Main features of computer algorithms for RIA data reduction. Comparison of some different approaches for the interpolation of dose–response curve. J Nucl Med Allied Sci 1982;26:235–48.

19. Clerico A, Ghione S, Del Chicca MG, Balzan S. Problems in standardization of digitalis-like substance assays by means of competitive immunological methods [Letter]. Clin Chem 1987;33:340–1.

20. Valdes Jr R, Hagberg JM. Exercise-induced elevation of endogenous digoxin-like immunoactive factor in human serum [Abstract]. Clin Chem 1985;31:1008.

21. Clerico A, Cambi A, Del Chicca MG, Cecchini L, Giaconi S. Urinary excretion of digoxin-like immunoreactivity after physical exercise [Letter]. Clin Chem 1988;34:215.

22. Balzan S, Ghione S, Clerico A, Montali U. Correlation between endogenous digoxin-like immunoreactivity and ^{3}H-ouabain displacement on erythrocyte membranes in extracts of human plasma. Clin Biochem 1986;19:311–4.

23. Deckert T, Feldt-Rasmussen B, Mathiesen ER, Baker L. Pathogenesis of incipient nephropathy: a hypothesis. Diabetic Nephropathy 1984;3:83–8.

24. De Fronzo RA. The effect of insulin on renal sodium metabolism. A review with clinical implications. Diabetologia 1981;21:165–71.

25. Vierhapper H. Effect of exogenous insulin on blood pressure regulation in healthy and diabetic subjects. Hypertension 1985;7(Suppl 2):49–53.

26. Johansen K, Hansen AP. High twenty-four-hour level of serum growth hormone in juvenile diabetes. Br Med J 1969;ii:356–7.

27. Biglieri EG, Watlington CO, Forsham PH. Sodium retention with human growth hormone and its subfractions. J Clin Endocrinol Metab 1961;21:361–70.

28. Deray G, Rieu M, Devynck MA, et al. Evidence of an endogenous digitalis-like factor in the plasma of patients with acromegaly. N Engl J Med 1987;316:575–80.

29. Das PK, Bray GM, Aguayo AJ, Rasminsky M. Diminished ouabain-sensitive, sodium-potassium ATPase activity in sciatic nerves in rats with streptozotocin-induced diabetes. Exp Neurol 1976;53:285–8.

30. Pierce G, Dhalla N. Sarcolemmal Na^{+}-K^{+}-ATPase activity in diabetic rat heart. Am J Physiol 1983;245:C241–7.

31. Jakobsen J, Knudsen GM, Juhler M. Cation permeability of the blood–brain barrier in streptozotocin diabetic rats. Diabetologia 1987;30:409–13.

32. Finotti P, Palatini P. Reduction of erythrocyte (Na^{+}-K^{+}) ATPase activity in Type 1 (insulin-dependent) diabetic subjects and its activation by homologous plasma. Diabetologia 1986;29:623–8.

33. Viberti GC, Keen H, Wiseman MJ. Raised arterial pressure in parents of proteinuric insulin dependent diabetes. Br Med J 1987;295:515–7.

34. Krolewski AS, Canessa M, Warram JH, et al. Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. N Engl J Med 1988;318:140–5.

35. Mangili R, Bending JJ, Scott G, et al. Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. N Engl J Med 1988;318:146–50.