

A Double-Blind, Placebo-Controlled, Crossover Trial Comparing the Effects of Amiloride and Hydrochlorothiazide on Glucose Tolerance in Patients With Essential Hypertension

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See Editorial Commentary, pp 911–912

Abstract—Hypertension guidelines advise limiting the dose of thiazide diuretics and avoiding combination with β -blockade, because of increased risk of diabetes mellitus. We tested whether changes in the 2-hour oral glucose tolerance test could be detected after 4 weeks of treatment with a thiazide and could be avoided by switching to amiloride. Two double-blind, placebo-controlled, crossover studies were performed. In study 1 (41 patients), we found that changes in glucose during a 2-hour oral glucose tolerance test could be detected after 4 weeks of treatment with bendroflumethiazide. In study 2, 37 patients with essential hypertension received, in random order, 4 weeks of once-daily treatment with hydrochlorothiazide (HCTZ) 25 to 50 mg, nebivolol 5 to 10 mg, combination (HCTZ 25–50 mg+nebivolol 5–10 mg), amiloride (10–20 mg), and placebo. Each drug was force titrated at 2 weeks and separated by a 4-week placebo washout. At each visit, we recorded blood pressure and performed a 75-g oral glucose tolerance test. Primary outcome was the difference in glucose (over the 2 hours of the oral glucose tolerance test) between 0 and 4 weeks, when HCTZ and amiloride were compared by repeated-measures analysis. For similar blood pressure reductions, there were opposite changes in glucose between the 2 diuretics ($P<0.0001$). Nebivolol did not impair glucose tolerance, either alone or in combination. There was a negative correlation between Δ potassium and Δ 2-hour glucose ($r=-0.28$; $P<0.0001$). In 2 crossover studies, 4 weeks of treatment with a thiazide diuretic impaired glucose tolerance. No impairment was seen with K^+ -sparing diuretic or β_1 -selective blockade. Substitution or addition of amiloride may be the solution to preventing thiazide-induced diabetes mellitus. (*Hypertension*. 2012;59:934-942.)

Key Words: hydrochlorothiazide ■ amiloride ■ glucose tolerance ■ hypertension

Thiazide diuretics were developed in the 1950s and have been used for the treatment of hypertension for several decades.¹ Outcome trials have shown cardiovascular benefits from the use of thiazides in essential hypertension, which are similar to those achieved with other antihypertensive agents.² However, there has been recent recognition that currently used doses are smaller than those used in most of the outcome trials.^{3–5} The main adverse effects limiting the use of thiazides are metabolic, including hypokalemia, hyponatremia, hyperuricemia, and hyperglycemia.^{1,6} Several long-term studies have shown an increased incidence of new-onset type 2 diabetes mellitus with use of thiazides.^{7–10} The mechanism of new-onset diabetes mellitus remains undetermined, and in 2007 the National Heart, Lung, and Blood Institute called for

prospective research into the role of reduced K^+ and whether prevention of hypokalemia would prevent hyperglycemia.^{11–13} Although the number of studies is fewer and they do not provide outcome data, the potassium-sparing diuretic amiloride has been shown to have comparable blood pressure-lowering efficacy to thiazides and, therefore, could be a useful addition to thiazides if (as suggested long ago) they have a neutral or beneficial effect on glucose tolerance.^{14,15}

β -Blocker therapy has also been associated with adverse effects on glucose metabolism, especially when used in combination with thiazides.² This has led to β -blockers being relegated to fourth-line treatment in some treatment algorithms for essential hypertension.^{5,16} The high dose of atenolol used in the Anglo-Scandinavian Cardiac Outcomes

Received December 8, 2011; first decision December 22, 2011; revision accepted March 14, 2012.

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Menarini had no involvement in the running of the study, data collection or analysis, or in writing the article.

This trial has been registered at www.clinicaltrials.gov (identifier NCT00380289).

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Hypertension is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.111.189381

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
Week	0	2	4	8	10	12	16	18	20	24	26	28	32	34	36					
Treatment		Thiazide		Washout		β-blocker		Washout		Combination		Washout		Placebo		Washout		Amiloride		
Study 1		BFZ				Atenolol				BFZ+Atenolol										
Study 2		HCTZ				Nebivolol				HCTZ+Nebivolol								Amiloride		
Dose																				
Study 1		5	10			50	100			2.5/25	5/50									
Study 2		25	50			5	10			25/5	5/10							10	20	
Measurements																				
BP		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓
OGTT		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓

Figure 1. Flow diagram for studies 1 and 2. The study designs were identical with the exception that study 2 included an additional arm with amiloride and was, therefore, longer in total duration. The thiazide and β-blocker used were also different in the 2 studies. The study drugs in both studies were given in random order. HCTZ indicates hydrochlorothiazide; BFZ, bendroflumethiazide.

Trial (ASCOT) would render it nonselective, and metabolic effects of β-1 selective β-blockers, such as nebivolol, have been shown to be favorable in the short term.^{17–19}

The initial reports of thiazide hyperglycemia considered this to develop slowly over several years, and new-onset diabetes mellitus itself has usually been reported at annual intervals during outcome trials.^{20,21} However, the oral glucose tolerance test (OGTT) has been used to demonstrate earlier changes, after 12 weeks.¹⁰ The primary aim of our first study was to determine whether the diabetogenic effect of thiazides could be quantified as early as 4 weeks, thus allowing a crossover comparison of K⁺-losing and K⁺-sparing diuretics. To maximize this possibility, we used the thiazide and dose (bendrofluazide 10 mg) that was first reported to cause glucose intolerance. The primary aim of the second study was to determine whether patients being treated for essential hypertension with the potassium-sparing diuretic amiloride have better glucose tolerance during a 2-hour, 75-g OGTT compared with patients taking the thiazide diuretic hydrochlorothiazide (HCTZ); the secondary aim was to determine whether a β-1 selective β-blocker (nebivolol) has a neutral or beneficial effect on glucose tolerance when used in monotherapy or in combination with HCTZ.

Methods

Subjects

Patients with essential hypertension, aged 18 to 75 years, were recruited from primary and secondary care. Ethics approval from a local research ethics committee was obtained, and all of the participants gave informed consent before commencing the study. The blood pressure entry criteria for the study were as follows: (1) untreated, blood pressure (BP) 140 to 170/90 to 110 mm Hg; (2) treated for >1 month with drugs other than β-blockers or diuretic and BP >140/85 mm Hg; or (3) treated for >1 month with drugs other than β-blockers or diuretic and BP <140/85 mm Hg and patient willing to change medication for the duration of the study. In study 2, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers were discontinued where possible at the start of the study. Patients with intolerances or contraindications to the study medications were excluded.

Design

Both studies were double-blind, placebo controlled crossovers (Figure 1). The active medications were given for 4 weeks, with forced titration (dose doubling) at 2 weeks. Study 2 included an additional arm with amiloride and, therefore, was of longer duration than study 1. The different study drugs (including amiloride) were given in random order in both studies. Each cycle of active treatment was separated by 4 weeks of single-blind placebo treatment. Patients attended the clinical investigation ward at 0, 2, and 4 weeks of each drug treatment. At each study visit, seated BP was measured (Omron), electrolytes were measured, and a 75-g OGTT was performed. Blood samples were also taken at each visit for fasting and 30-minute insulin concentrations.

Drug Treatment

Study 1

The primary aim was to examine whether 4 weeks of treatment with a thiazide diuretic was sufficient to cause detectable changes during a 2-hour, 75-g OGTT. The secondary aim was to determine whether a similar change occurs during β-blockade and whether, on a combination of both β-blockade (atenolol) and thiazide, there was an additional effect on OGTT compared with that seen with either drug alone. The study drugs chosen were the most commonly prescribed diuretic (bendroflumethiazide; BFZ) and β-blocker (atenolol) in the United Kingdom at the time of study. The doses of atenolol were those used in the ASCOT (50–100 mg). We used the same diuretic as ASCOT but at higher doses (BFZ, 5–10 mg). This was the current practice when the hyperglycemic effects of thiazides were first detected.²⁰ We expected rapid metabolic changes to be most likely at higher doses.

Study 2

The primary aim of study 2 was to determine whether patients receiving antihypertensive treatment with the potassium-sparing diuretic amiloride have better glucose tolerance during a 2-hour, 75-g OGTT compared with the thiazide diuretic, HCTZ. The secondary aims were to evaluate effects of a more β₁-selective blocker than atenolol on glucose tolerance, both as a single agent and in combination with thiazide. This time, we used HCTZ 25 mg increasing to 50 mg, which we expected to reproduce the effects of BFZ. For β-blockade, we used the most β₁-selective agent, nebivolol (5 mg increasing to 10 mg OD). The combination used HCTZ/nebivolol at the same doses as in the monotherapy cycles with these agents. Amiloride was prescribed at 10 mg OD increasing to 20 mg OD.

Table 1. Baseline Characteristics of Patients in Study 1 and Study 2

Variable	Study 1	Study 2
Sex, male/female	29/12	17/20
Age, y	60 (35–74)*	65 (41–75)*
BMI, kg/m ²	29.4 (5.1)	28.6 (4.2)
Baseline SBP, mm Hg	141.7 (14.8)	144.3 (13.7)
Baseline DBP, mm Hg	87.0 (10.2)	85.4 (9.9)
No. of subjects requiring background antihypertensives, none/CCB/ACE or ARB/ α -blocker†	8/20/20/5	7/22/6/5
No. of subjects requiring background antihypertensives, 1 drug/2 drugs/3 drugs	18/14/1	28/1/1

Data are mean (SD) unless otherwise specified. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

*Data are median (range).

†Some subjects required >1 class of antihypertensive drug.

Statistical Methods

Power Calculations

For study 1, the primary outcome was the change from baseline in glucose concentrations during a standard 2-hour, 75-g OGTT after 4 weeks of treatment with BFZ. For study 2, the primary outcome was the change in glucose concentrations during a standard 2-hour, 75-g OGTT after 4 weeks of treatment with HCTZ compared with 4 weeks of treatment with amloride.

To estimate the likely change in glucose, during thiazide therapy and within-subject SD, a pilot study of 10 patients was performed. From this we calculated that 34 patients would give 90% power at $\alpha=0.01$ to detect a difference in 2-hour glucose between 7.3 and 7.8 mmol/L (SD 0.9). We aimed to recruit 40 patients in each study to allow for discontinuations from the crossover.

Data Presentation and Analysis

The data are presented as mean (SD) or median (range). The changes in glucose concentrations over the 2-hour OGTT between drugs and between visits were analyzed by repeated-measures analysis. Between-subjects variables included background treatment. Single-point comparisons between baseline and 4 weeks were undertaken by paired *t* tests. Primary and secondary outcomes were determined using data from the weeks 0 and 4 of each cycle. Exploratory analyses included inspection of dose-trends and used data from 0, 2, and 4 weeks to look for correlations between variables by Pearson

correlation analysis. All of the analyses were performed using SPSS version 17.0.

Results

Study 1

Baseline Characteristics

There were 41 patients. Mean baseline systolic BP was 141.7 mm Hg (14.8 mm Hg), and mean baseline diastolic BP was 87.0 mm Hg (10.2 mm Hg) (Table 1).

Glucose Tolerance

Changes in Glucose During OGTT After 4 Weeks of Treatment With Each Drug Compared With Baseline

After 4 weeks of treatment with BFZ, there was a significant increase in the blood glucose during the 2-hour OGTT ($P<0.006$ versus baseline), with a trend already apparent after 2 weeks of 5 mg (Figure 2). There were also point increases in the fasting and 2-hour glucose after BFZ therapy (Table 2). Glucose concentrations during the 2-hour OGTT with 4 weeks of treatment with atenolol were unchanged compared with baseline, with a numeric trend toward improvement ($P=0.056$ versus baseline). There was no significant change in repeated-measures glucose concentrations during the 2-hour OGTT between baseline and 4 weeks of BFZ/atenolol combination treatment ($P=0.27$) or between BFZ monotherapy and BFZ/atenolol combination therapy over the 4-week treatment period ($P=0.30$). There were also no significant point differences between the 2-hour glucose between BFZ monotherapy and BFZ combination therapy with atenolol ($P=0.81$).

Other Biochemical Changes During the Study

Changes in 30-minute insulin and K⁺ between baseline and 4 weeks of treatment are shown in Table 2, as well as the point changes in fasting and 2-hour glucose during OGTT. BFZ, alone and in combination with atenolol, reduced serum K⁺, but there were no significant changes in insulin.

Changes in BP Between Baseline and 4 Weeks of Treatment

As expected, systolic BP and diastolic BP fell on all active treatments, with the greatest BP reduction seen with BFZ/atenolol combination therapy (Table 2).

Table 2. Results for Study 1 Comparing Results Between Baseline and 4-Weeks Treatment for Each Drug

Variable	Atenolol		BFZ		Combination (BFZ/Atenolol)		Placebo	
	0 wk	4 wk	0 wk	4 wk	0 wk	4 wk	0 wk	4 wk
Fasting glucose, mmol/L	5.34 (0.74)	5.38 (0.76)	5.38 (0.62)	5.64 (0.76)*†	5.46 (0.72)	5.73 (0.88)*†	5.34 (0.66)	5.36 (0.66)
2-h glucose, mmol/L	7.78 (2.52)	7.42 (2.14)	7.48 (2.62)	8.35 (2.89)*‡	7.76 (2.77)	8.19 (3.05)‡	7.65 (2.58)	7.87 (2.66)
Fasting insulin, pmol/L	74.8 (56.6)	69.4 (62.7)	75.4 (48.6)	91.9 (67.1)	81.1 (60.6)	77.9 (68.3)	75.4 (51.1)	70.5 (60.9)
30 min insulin, pmol/L	430.4 (338.3)	433.8 (452.0)	492.2 (517.4)	535.4 (478.2)	454.1 (410.2)	466.9 (430.6)	433.9 (348.6)	463.1 (335.3)
K ⁺ , mmol/L	4.1 (0.2)	4.2 (0.3)	4.1 (0.3)	3.6 (0.4)*	4.1 (0.3)	3.8 (0.4)*	4.1 (0.3)	4.1 (0.3)
SBP, mm Hg	139.4 (12.9)	127.6 (16.3)*	138.6 (11.3)	129.4 (10.4)*	140.8 (12.3)	120.2 (10.2)*	139.3 (13.3)	137.8 (12.9)
DBP, mm Hg	85.9 (9.0)	77.3 (8.0)*	86.1 (9.6)	81.0 (6.5)*	87.0 (9.8)	74.4 (8.6)*	86.1 (10.5)	85.1 (9.2)

Results are shown as mean (SD). BFZ indicates bendroflumethiazide; K, plasma potassium; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* $P<0.001$.

†There was no significant difference between fasting glucose for BFZ monotherapy and BFZ/atenolol combination therapy with nebivolol ($P=0.23$).

‡There was no significant difference between 2-h oral glucose tolerance test glucose for BFZ monotherapy and BFZ combination therapy with nebivolol ($P=0.81$).

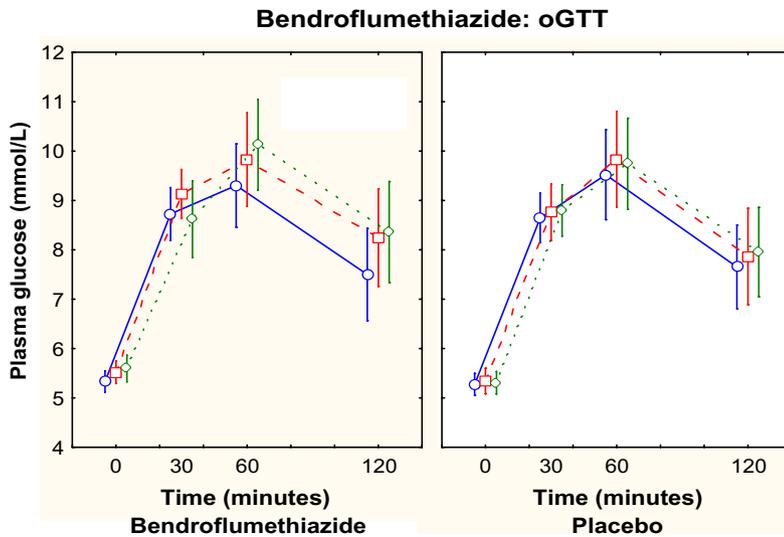


Figure 2. Oral glucose tolerance test (OGTT) in study 1 comparing bendroflumethiazide (BFZ; left panel) with placebo (right panel) after 0, 2, and 4 weeks of dosing. Values are mean±SE ($P=0.006$ for changes in the OGTT comparing BFZ with baseline over the 4-week study period). ○, week 0; □, week 2 (BFZ 5 mg); ◇, week 4 (BFZ 10 mg).

Study 2

Baseline Characteristics

Thirty-seven patients completed the crossover. Mean baseline systolic BP was 144.3 mm Hg (13.7 mm Hg), and mean baseline diastolic BP was 85.4 mm Hg (9.9 mm Hg) (Table 1). In this study, only 2 patients received >1 background treatment, and we were able to withdraw angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in all but 6 patients.

Changes in Overall OGTT Glucose Over 4-Week Study Period: HCTZ Versus Amiloride

The glucose increase during the 2-hour OGTT rose after 4 weeks of treatment with HCTZ; there was no change in the OGTT after treatment with amiloride (Figure 3). The difference in response to the 2 diuretics was highly significant in the planned primary comparison by repeated-measures ANOVA ($P<0.0001$). Adjusting for background medication,

including for concomitant treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, had no effect on the results. Once again, the lower dose of thiazide had an apparent effect in just 2 weeks.

Changes in OGTT Glucose Over 4-Week Study Period: Study Drugs Versus Placebo

Results for the OGTT glucose for the individual study drugs versus placebo are shown in Figure 4. Compared with the well-replicated OGTT at the end of 1 month of placebo, the rise in glucose concentrations during the 2-hour OGTT was augmented by HCTZ, either as monotherapy ($P<0.0001$ vs placebo) or HCTZ in combination with nebivolol ($P=0.017$ versus placebo). There was no difference in glucose concentrations during the 2-hour OGTT between HCTZ monotherapy and HCTZ/nebivolol combination therapy over the 4-week treatment period ($P=0.091$). The trends toward a diminished OGTT response after amiloride or nebivolol were not significant (both $P>0.30$ compared with placebo). However, compared with HCTZ, the effect of nebivolol on OGTT was highly significant ($P<0.0001$).

Other Biochemical Changes

Glucose

There was an increase in fasting glucose between baseline and 4 weeks in the HCTZ and HCTZ/nebivolol combination therapy arms of study 2. There were no significant changes in the other arms of the study (Table 3). Point changes at 2 hours are also shown in Table 3.

Insulin

Thirty-minute insulin rose numerically in all arms of the study between baseline and 4 weeks. This reached statistical significance only for amiloride ($P<0.001$). The difference (between baseline and 4 weeks) in the 30-minute rise in insulin was greater with amiloride compared with any other treatment, HCTZ ($P=0.005$), HCTZ/nebivolol combination therapy ($P=0.003$), nebivolol ($P=0.01$), and placebo ($P=0.045$). There were no significant treatment effects on fasting insulin except for the greater rise in fasting insulin on

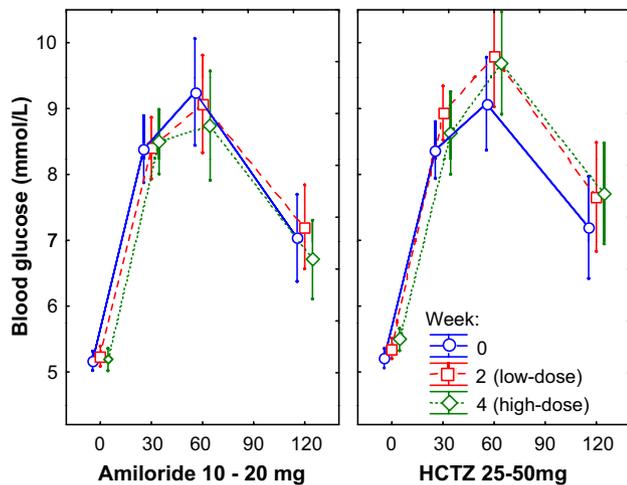


Figure 3. Oral glucose tolerance test (OGTT) in study 2 comparing amiloride (left panel) with hydrochlorothiazide (right panel) after 0, 2, and 4 weeks of dosing. Values are mean±SE ($P<0.0001$ for changes in the OGTT comparing amiloride with HCTZ over 4 weeks). ○, week 0; □, week 2 (low-dose); ◇, week 4 (high-dose).

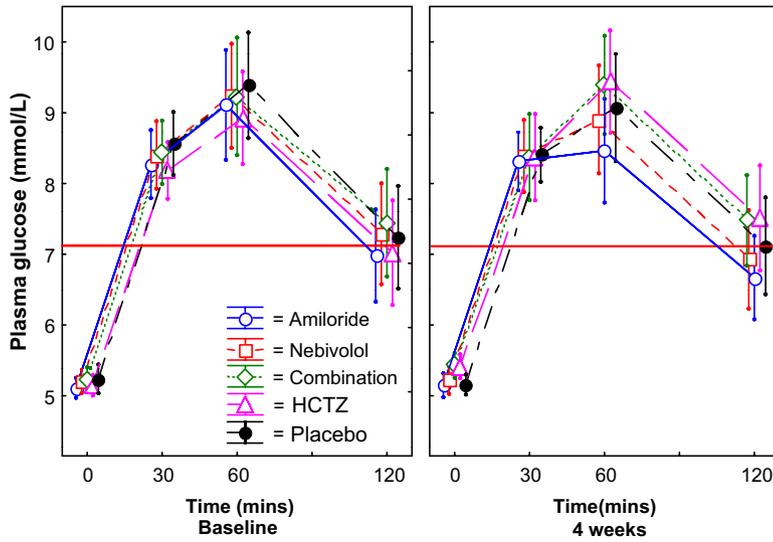


Figure 4. Glucose concentrations during the oral glucose tolerance test (OGTT) in study 2 comparing all of the study drugs and placebo at 0 and 4 weeks. Values are mean±SE. The red horizontal line is drawn through the placebo values. Combination therapy was with hydrochlorothiazide (HCTZ) plus Nebivolol. ○, Amiloride; □, Nebivolol; ◇, combination; ●, placebo; △, HCTZ.

combination therapy between baseline and 4 weeks ($P=0.047$; Table 3).

Potassium: Changes in Plasma Potassium Concentrations Between Baseline and 4 Weeks of Treatment

There were the expected falls between baseline and 4 weeks of treatment on HCTZ and combination therapy and rise in plasma potassium on amiloride. There was no change in plasma potassium on nebivolol (Table 3).

Blood Pressure

All of the active treatments reduced BP, with the greatest reduction on combination therapy (Table 3 and Figure 5). The BP-lowering effect of amiloride was numerically lower than that of HCTZ, but there were no statistically significant differences in systolic BP or diastolic BP between HCTZ and amiloride over the 4-week treatment period ($P=0.23$ and $P=0.82$, respectively; Figure 5). There were no correlations between change in systolic BP over 4 weeks of treatment and change in the OGTT 2-hour glucose over the same period, either in individual studies or in the 2 studies combined.

Exploratory Analysis of Correlations Among the Changes in Glucose, Potassium, and Insulin

There was a negative correlation between the change in plasma potassium over 4 weeks of treatment and change in the 2-hour glucose over this period, for study 1 ($r=-0.34$; $P<0.0001$), study 2 ($r=-0.28$; $P<0.0001$), and both studies combined ($r=-0.32$; $P<0.0001$; Figure 6). No correlations were found between the changes in plasma potassium and fasting glucose. There was a smaller positive correlation between the change in plasma potassium over 4 weeks of treatment and the change in 30-minute insulin over the same period, in study 2 ($r=0.173$; $P=0.03$), and in both studies combined ($r=0.143$; $P=0.01$).

Discussion

These studies show that the well-documented impairment by thiazide diuretics of glucose tolerance develops within a few weeks, with similar increases in the 2-hour glucose seen after either bendroflumethiazide 10 mg or HCTZ 50 mg. By contrast, treatment with a K^+ -sparing diuretic did not impair

Table 3. Results for Study 2 Comparing Results Between Baseline and 4-Weeks Treatment for Each Drug

Variable	Amiloride		Nebivolol		HCTZ		Combination (HCTZ/Nebivolol)		Placebo	
	0 wk	4 wk	0 wk	4 wk	0 wk	4 wk	0 wk	4 wk	0 wk	4 wk
Fasting glucose, mmol/L	5.19 (0.47)	5.23 (0.55)	5.23 (0.52)	5.24 (0.59)	5.16 (0.52)	5.45 (0.55)†‡	5.25 (0.63)	5.47 (0.64)*‡	5.30 (0.71)	5.19 (0.52)
2-h glucose, mmol/L	7.07 (2.17)	6.70 (1.88)	7.21 (2.09)	6.79 (2.21)	7.00 (2.36)	7.55 (2.24)*§	7.39 (2.44)	7.65 (2.13)§	7.38 (2.38)	6.65 (2.08)*
Fasting insulin, pmol/L	66.4 (46.6)	65.5 (31.5)	61.5 (38.1)	66.9 (44.6)	67.1 (38.3)	71.8 (39.7)	64.2 (47.2)	75.6 (48.4)*	68.0 (43.0)	61.1 (34.9)
30-min insulin, pmol/L	391.2 (297.4)	467.8 (267.6)†	373.3 (250.6)	395.8 (257.8)	381.2 (223.6)	383.2 (253.5)	389.8 (221.4)	420.8 (322.8)	385.2 (220.1)	387.6 (225.8)
K^+ , mmol/L	4.1 (0.3)	4.5 (0.3)†	4.2 (0.3)	4.3 (0.3)	4.2 (0.3)	3.7 (0.4)†	4.1 (0.3)	3.7 (0.3)†	4.2 (0.4)	4.1 (0.3)*
SBP, mm Hg	139.1 (12.9)	132.3 (15.0)†	142.3 (14.7)	131.2 (16.2)†	140.6 (12.0)	129.8 (13.7)†	140.6 (16.0)	121.6 (12.2)†	141.0 (11.4)	137.0 (11.8)*
DBP, mm Hg	82.8 (8.4)	81.2 (7.8)	84.4 (11.5)	77.4 (10.4)†	84.1 (8.7)	80.7 (9.8)*	83.8 (10.0)	74.7 (9.8)†	85.6 (8.1)	83.1 (8.6)*

Data are mean (SD). HCTZ indicates hydrochlorothiazide; K, plasma potassium; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* $P<0.05$.

† $P<0.001$.

‡There was no significant difference between fasting glucose for HCTZ monotherapy and HCTZ/nebivolol combination therapy with nebivolol ($P=0.72$).

§There was no significant difference between 2-h oral glucose tolerance test glucose between HCTZ monotherapy and HCTZ/nebivolol combination therapy with nebivolol ($P=0.42$).

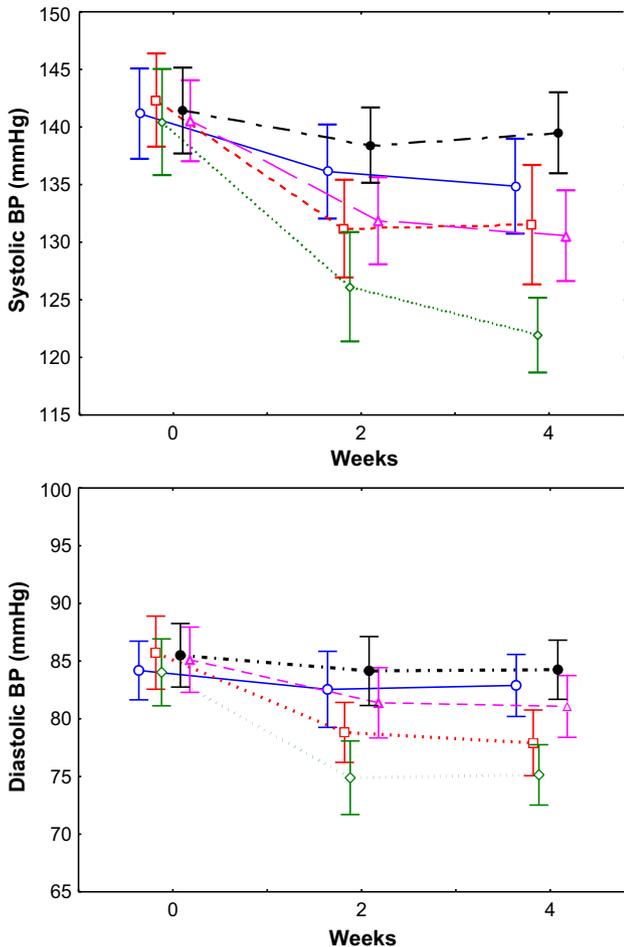


Figure 5. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) at 0, 2, and 4 weeks for each arm of study 2. Values are mean \pm SE. \circ , Amiloride; \square , Nebivolol; \diamond , combination; \bullet , placebo; \triangle , hydrochlorothiazide (HCTZ).

glucose tolerance; and β_1 -selective blockade was also neutral, both on its own and in combination with HCTZ. Although these results were largely as predicted, there have not been previous prospective randomized comparisons of K^+ -losing and K^+ -sparing diuretics, which investigated glucose tolerance; and even 50 years after the first use of diuretics for hypertension there is a resurgence of interest and uncertainty concerning the optimal diuretic regime.

The novelty of, and necessity for, study 1 was its demonstration that a transient, significant increase in blood glucose would be induced by a period of thiazide dosing that permitted a comparison of several drug classes within a crossover trial. Only a minority of patients within the outcome trials develop diabetes mellitus, and in only a minority of these does the diabetes mellitus appear drug induced. It was important, therefore, to discover whether OGTT would enable a rapid signal to be detected within a small cohort of hypertensive patients. The thiazide and dose were selected as those first linked to the metabolic effects of thiazides during the OGTT²⁰; in a mechanistic study we also considered it appropriate to use the intervention most likely to induce the metabolic changes that we wished to investigate. Although bendroflumethiazide 10 mg is a high dose by current stan-

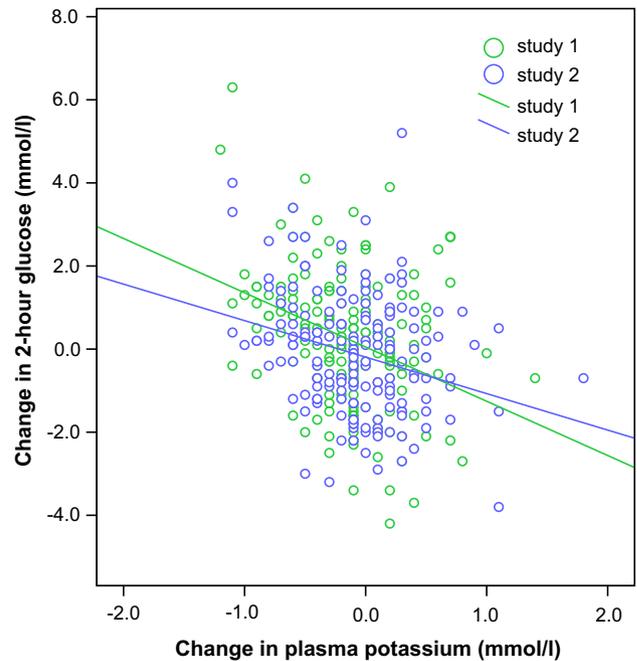


Figure 6. Inverse correlation of the change in plasma potassium, between baseline and 4 weeks, with the change in 2-hour glucose over the same period. The graph is a scatterplot showing the values for each subject on each treatment in study 1 ($r = -0.34$; $P < 0.0001$) and study 2 ($r = -0.28$; $P < 0.0001$). Green circle, study 1; blue circle, study 2; green line study 1; blue line, study 2.

dards, it is the dose that prevents stroke during long-term treatment, and a recent meta-analysis and review of national guidance of antihypertensive therapy have called into question the efficacy of lower dose thiazides.^{5,22,23} It was in part concern about the diabetogenic effect of higher doses that led to their disuse and that needs now to be overcome if effective diuretic dosing in hypertension is to be resumed. A secondary aim of study 1 was to observe whether the presumed additive risk of diuretic and blockade for development of diabetes mellitus could be supported by an additive effect on short-term changes in glucose tolerance.^{8,24} The outcome trials in which both classes were used were not designed to permit additivity to be proven, and no evidence for this was found in our study. Indeed, there was a hint that glucose tolerance might even improve when atenolol was studied at a lower, more β_1 -selective dose than the average dose of atenolol in ASCOT or The Losartan Intervention for Endpoint Reduction (LIFE) in Hypertension Study.^{8,25}

Having found that a short-term change in glucose tolerance could be used as primary outcome measure of thiazide action, we designed a second, similar study to ask primarily whether the effect on glucose could be avoided with a K^+ -sparing diuretic and, secondarily, whether the neutral or beneficial influence of β_1 -selective blockade could be borne out using a more selective β -blocker than atenolol. This time we used HCTZ rather than bendroflumethiazide, because it is the most widely used thiazide diuretic in clinical practice worldwide. The lower, 25 mg, dose of HCTZ is in common use, whereas the higher dose was used in two thirds of patients randomized to diuretic in the Intervention as a Goal in Hypertension

Treatment Study, where HCTZ was associated with a 25% excess of new-onset diabetes mellitus.⁹ As a K⁺-sparing diuretic we used amiloride, which (unlike spironolactone) has been used together with HCTZ in 2 outcome trials in hypertension.^{9,26} However, we used a much higher dose of amiloride than the 2.5 to 5.0 mg that is routinely added to HCTZ in some generic formulations and that was inadequate to negate the glycemic action of HCTZ in the Intervention as a Goal in Hypertension Treatment Study.⁹ We have previously studied amiloride 20 mg and 40 mg in crossover comparisons with bendroflumethiazide and spironolactone and found them approximately equivalent to BFZ 5 mg in reducing BP in patients with low-renin hypertension.^{15,27}

The difference in glucose tolerance between HCTZ and amiloride seemed attributed mainly to the expected hyperglycemic action of HCTZ, with both doses of HCTZ causing similar rises in glucose during OGTT. Although there was a trend for amiloride to blunt the glucose response, there was no significant benefit compared with placebo. Although previous studies have shown similar BP-lowering efficacy of high-dose amiloride to other diuretics,¹⁵ these also confirmed the observation in the first clinical study of amiloride that its comparable natriuretic efficacy to spironolactone leads to a brisk rise in renin and aldosterone, which probably blunt the fall in BP.²⁸ We frequently, therefore, use amiloride in the clinic in combination with blockers of the renin-angiotensin system. In the present study, such drugs were withdrawn from most patients at screening because of the possibilities that they themselves influence glucose tolerance or lead to hyperkalemia in a blinded study using forced dose titration of amiloride. In the clinic, where a dose of amiloride 40 mg would require 8 tablets rather than a single overencapsulation, 20 mg is the maximum plausible dose until or unless its wider use leads to new formulations.

The mechanism by which thiazides impair glucose metabolism is not understood and could be secondary to induction of either or both peripheral insulin resistance and impaired β -cell function.^{11,29} We found a rise in fasting insulin on thiazide, which suggests that thiazides may increase insulin resistance, and the results for amiloride showed the converse. The rise in 30-minute insulin on all of the therapies was significant only on amiloride, where it was greater than in the other treatment arms. This suggests that β -cell function may improve on amiloride treatment, whereas compensatory β -cell responses to overcome insulin resistance induced by thiazides were inadequate to prevent a rise in plasma glucose. Interestingly, a previous study in mice injected with amiloride showed a significant increase in serum insulin concentration and hypoglycemia, and also the release of insulin was increased when isolated mouse islets were exposed to 1 or 2 mmol/L of amiloride.³⁰

We demonstrated a negative relationship between change in potassium concentration and change in 2-hour glucose concentration over the 4-week study period (Figure 6). However, like Smith et al, we did not find a relationship between change in potassium concentration and change in fasting glucose concentration over the 4-week study period.³¹ Previous studies have also suggested that thiazide-induced hypokalemia may affect glucose metabolism, but this hypoth-

esis is controversial, because patients with chronic hypokalemia attributed other causes, such as Conn's syndrome and Gitelman's syndrome, do not have a clearly higher incidence of diabetes mellitus.^{11,12,29,32} It may well be that a reduction in both insulin sensitivity and plasma K⁺, impairing insulin secretion, is required to impair glucose tolerance. Our findings are, thus, consistent with the consensus that thiazides cause insulin resistance, and (with less evidence) that K⁺-sparing diuretics avoid glucose intolerance through enhancing insulin secretion.^{33,34} There are not, to our knowledge, any randomized studies investigating the effects of spironolactone, another potassium-sparing anti-hypertensive, on glucose tolerance. Previous small studies have shown variable effects of spironolactone, with a transient decrease, no change, or improvement in insulin sensitivity being demonstrated.^{26,35,36}

Our studies suggest that, at β_1 -selective doses, β -blockade does not impair glucose tolerance or exacerbate the effect of thiazides. Because the pancreatic β -adrenoreceptor in humans is β_2 , it seems likely that the apparent additivity of thiazides and β -blockade (on risk of new-onset diabetes mellitus) in trials like ASCOT and The Losartan Intervention for Endpoint Reduction (LIFE) in Hypertension Study was attributed to the frequent use of atenolol 100 mg.^{8,25} With a selectivity ratio of <10, high-dose atenolol is likely to block insulin secretion.³⁷

Previous Comparisons

Few previous studies have directly compared the relative effects of thiazide and potassium-sparing diuretics on glucose metabolism. Thomas and Thompson¹⁴ showed that glucose tolerance returned to normal in 2 patients on long-term HCTZ who were changed to amiloride but showed no changes in glucose metabolism in patients newly commenced on amiloride. The study showed equivalent BP lowering on either diuretic. Other studies have also found equivalent BP-lowering efficacy between thiazides and potassium-sparing diuretics.^{15,27}

Previous studies investigating effects of β -1 selective β -blockers on glucose metabolism have found the effect to be neutral or beneficial.^{17,18} A study investigating the effect of adding HCTZ to nebivolol monotherapy showed an initial 26% improvement in insulin sensitivity with nebivolol alone, which was blunted by the addition of HCTZ. This study showed improvement in BP with the addition of the diuretic.¹⁹

Potential Limitations of Study

This study was of relatively short duration, and changes seen over 4 weeks treatment may not predict long-term changes in glucose metabolism. Nevertheless, impairment of glucose metabolism during the OGTT, especially when combined with the fasting and first-phase insulin responses, are a good, arguably the best, predictor for type 2 diabetes mellitus.^{10,38,39} We used OGTT as our primary outcome measure because, in a multiple crossover study, a relatively simple and well-tolerated procedure is required to retain subject participation. Glucose-clamp techniques may be useful in further studies to investigate the pathophysiological mechanisms.⁴⁰

Crossover studies are susceptible to criticisms of carryover effects between treatment and small size overall. The former can be countered by randomization of order and inclusion of order as a factor in the ANOVA. Traditionally, the main reasons for undertaking crossovers are the potential they offer for single center studies and for investigating variability in response to drugs. But, in addition, recent recognition of the enormous molecular complexity of hypertension explains why it has been easier to demonstrate clear-cut differences between drug classes in small crossovers compared with much larger parallel groups comparisons.^{41–43} With an expectation that several hundred genetic variants contribute to hypertension, only the largest studies can expect similar genetic susceptibilities in each group. The difference in absolute risk of new-onset diabetes mellitus between thiazide and comparator drugs in the outcome trials is <1% per year. Although a retrospective analysis of the individual blood glucose data in the Intervention as a Goal in Hypertension Treatment Study showed an upward trend over time in most subjects (M.J. Brown, unpublished data, 1998), the slope of the trend was variable, and much smaller parallel-group comparisons could easily be thrown by imbalanced randomization.

Perspectives

The small size of crossover trials limits their immediate impact on clinical practice. However, as commented above, the evidence base for current practice may be weaker than long assumed. In particular, the use of low-dose thiazides has come into question, both in a recent meta-analysis and in the recent revision of the United Kingdom National Institute for Health and Clinical Excellence hypertension guidance.^{5,23} It is, therefore, timely to consider whether there is to be a change from using low-dose thiazide and whether this should be to a thiazide-like diuretic or to a combination with a potassium-sparing diuretic. Unlike low-dose thiazide, such combinations have been shown to be effective in outcome trials.^{9,26} However, commercially available combinations of thiazide and K⁺-sparing diuretic use much lower doses of the latter than we have investigated. Therefore, a 500-patient multisite study, Prevention and Treatment of Hypertension With Algorithm-Based Therapy 3, funded by the British Heart Foundation, is now directly comparing the effects of HCTZ and high-dose amiloride on glucose tolerance and has included a third group randomized to half-dose of each.

In summary, rapid onset of changes in the OGTT, after initiation of thiazide treatment, allowed a highly significant difference to be detected between K⁺-losing and K⁺-sparing diuretics. Recent recognition that low-dose thiazides do not, after all, provide the same long-term vascular protection as higher-dose thiazides prompts consideration and investigation of whether the addition of high-dose amiloride is a preferable alternative to doubling currently used doses of thiazide.

Acknowledgments

We acknowledge all of the patients who took part in the studies and the staff of the Clinical Investigation Ward and the biochemistry laboratory at Addenbrooke's Hospital.

Sources of Funding

This study was partially funded by an unrestricted grant from Menarini.

Disclosures

None.

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