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Effect of spironolactone and vitamin E on serum metabolic parameters and insulin resistance in patients with nonalcoholic fatty liver disease

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Abstract

Aim: The renin–angiotensin–aldosterone system has been implicated in the pathogenesis of insulin resistance and nonalcoholic fatty liver disease (NAFLD). The beneficial effect of spironolactone in a mouse model with diabetes and NAFLD has recently been reported. The main aim was assessment of the effect of spironolactone on serum metabolic parameters and insulin resistance in patients with NAFLD.

Methods: This study includes preliminary results of a single-centre randomised controlled trial of treatment with vitamin E (group 1, 10 patients) versus spironolactone plus vitamin E (group 2, 10 patients) in biopsy-proven NAFLD. Serum transaminases, lipids, potassium, sodium, glucose and insulin were measured, and homeostatic model assessment-insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated before and 8 weeks after baseline assessment.

Results: Insulin was decreased within group 2 (15.3 ± 2.7 at baseline vs. 10.3 ± 5.0 at week 8, $p = 0.013$). Although no difference in glucose was observed, HOMA-IR significantly decreased (4.4 ± 0.9 vs. 2.8 ± 0.5, respectively, $p = 0.047$). QUICKI was increased, but not statistically significantly.

Conclusions: Spironolactone and vitamin E combined therapy seems to exhibit a favourable effect on serum insulin and HOMA-IR in patients with NAFLD. If validated in a large-scale clinical trial, it may prove an inexpensive therapeutic approach for the management of NAFLD patients.

Keywords

Insulin resistance, metabolic syndrome, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, spironolactone, vitamin E

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a rapidly emerging chronic liver disease and is reported to affect up to 70–80% of overweight and obese individuals; it is the most common cause of hepatic dysfunction encountered in general practice and its incidence in both adults and children is rising, linked with age and burgeoning epidemics of obesity and type 2 diabetes mellitus (T2DM).[1,2] NAFLD represents a wide spectrum of liver damage ranging from simple nonalcoholic fatty liver (NAFL) to a more severe and treatment-resistant stage that features steatosis plus inflammation, termed nonalcoholic steatohepatitis (NASH), which may in turn progress to hepatic fibrosis, cirrhosis, and sub-acute liver failure. Insulin resistance (IR) is thought to play an important role in the pathogenesis of NAFLD, so that NAFLD is considered the hepatic component of IR or metabolic syndrome.[3]

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Unlike other chronic liver diseases (for example, hepatitis C), there are no effective treatment strategies for NAFLD. Currently, the management of NAFLD includes modification of underlying risk factors, detection of patients that have progressed to cirrhosis, management of cirrhosis-related morbidity and transplantation in patients with end-stage liver disease.[4] Diet, exercise, bariatric surgery and pharmacological treatment, including weight loss agents, insulin sensitisers, lipid-lowering agents, ursodeoxycholic acid and vitamin E have been investigated with some promising results.[4,5] Although vitamin E may benefit patients with NAFLD,[6,7] we believe that a single agent could hardly be an effective treatment, given the multifactorial pathogenesis of NAFLD.[3] Therefore, clinical trials should be directed towards the effect of multifactorial interventions, as recommended elsewhere.[8,9]

The renin–angiotensin–aldosterone system (RAAS) has been implicated in the pathogenesis of IR and NAFLD.[5,10,11] Recently, low-dose (25–50 mg/day) aldosterone antagonists in patients with heart failure diminish mortality, possibly by reducing cardiac and vascular fibrosis.[12] Moreover, the beneficial effect of 8-week spironolactone in a mouse model with diet-induced diabetes and NAFLD has been reported.[13] However, to our knowledge, the role of spironolactone in NAFLD patients has not yet been investigated. We are currently running a 52-week randomised controlled trial (RCT) in patients with biopsy-proven NAFLD assigned to receive vitamin E or spironolactone plus vitamin E. Our primary aim is to assess the effect of spironolactone on serum levels of adipokines at 52 weeks post-treatment period. After the report of the above-mentioned 8-week beneficial effect of spironolactone on an experimental model,[13] we performed an interim analysis in our clinical data; thus this study includes the 8-week preliminary results of vitamin E versus spironolactone plus vitamin E treatment in adults with histologically documented NAFLD.

Materials and methods

Eligible adults (≥ 18 years of age) were identified and recruited on an outpatient basis. Determination of eligibility was based on standard tests and procedures completed during screening. Each patient provided an informed consent at the screening visit to obtain any tests and procedures needed to finalise eligibility, and had a history and physical examination to identify other illness and contraindications for participation. The study protocol was approved by the ethics committee of Aristotle University of Thessaloniki. All the participants provided an informed consent. Inclusion criteria were: (a) bright liver on ultrasound imaging and increased liver function tests for at least 6 months before liver biopsy; and (b) biopsy-proven NAFLD (either NAFL or NASH) according to NAFLD Activity Score (NAS).[14] Exclusion criteria were generally the same as those proposed for PIVENS trial design,[15] with two modifications: (a) known intolerance to spironolactone as an exclusion criterion; and (b) the inclusion of patients with T2DM not receiving thiazolidinediones or insulin. Specifically, patients with ethanol consumption >20 g/day, history of liver disease (chronic viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, haemochromatosis, Wilson's disease and α1-antithrypsin deficiency), previous exposure to hepatotoxic drugs or evidence (clinical or histological) of liver cirrhosis were excluded. Eligible patients were randomised to receive either vitamin E (400 IU/day) (group 1) or spironolactone (25 mg/day) plus vitamin E (400 IU/day) (group 2) per os for 52 weeks. Randomisation was accomplished by a computer program before the screening of the first patient.

The primary aim of this RCT is assessment of the 52-week post-treatment effect of spironolactone plus vitamin E versus vitamin E on serum levels of adipokines. Apart from the screening visit and baseline visit, three more visits have been planned during the treatment phase (at 8, 26 and 52 weeks), as well as a visit after a 26-week washout period at the end of the treatment phase. For the report presented here, preliminary biochemical data from baseline visit and visit 2 (week 8) were analysed.

At baseline, a complete medical history, physical examination, serum samples and liver biopsy were obtained. Liver biopsy was performed under computed tomography guidance by an experienced radiologist (EZ) and interpreted by two experienced pathologists (KP, EK) who were blinded to the medications (vitamin E or spironolactone plus vitamin E) that the patients were assigned to receive. Morning (8–9 am) fasting serum samples were collected before liver biopsy. The following visits included medical history, physical examination, pill count and serum sample collection. Serum aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), cholesterol, triglycerides, high-density lipoprotein (HDL), potassium and sodium and glucose were measured within 1 h after blood was drawn, with standard methods using an automated analyser (Olympus AU2700; Olympus, Hamburg, Germany). Sera were also immediately frozen at -70°C for the measurement of adipokines and hormones at the end of the study. For the purposes of this report, serum was defrosted and insulin was measured with two-site immunochemiluminescence assay (ICMA) by Immulite 2500 immunoassay system (Siemens Healthcare Diagnostics, Los Angeles, CA, USA; intra-assay coefficient of variation (CV) 3.3–5.5%, total CV 4.1–7.3%).

Body mass index (BMI) was calculated by the formula body weight (kg) / height^2 (m). Low-density lipoprotein (LDL) was calculated with the Friedewald formula. To quantify insulin resistance, two indexes were used; homeostatic model assessment-insulin resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI), calculated by the formulas:

$$\text{HOMA-IR} = (\text{glucose [mmol/l]} * \text{insulin [}\mu\text{U/ml]}) / 22.5;[16]$$

$$\text{QUICKI} = 1 / (\log(\text{glucose}) \text{ [mg/dl]} + \log(\text{insulin}) \text{ [}\mu\text{U/ml]}).[17]$$

Statistical analysis

Continuous data are presented as mean ± standard error of the mean (SE). Between or within-group comparisons were performed by non-parametric tests, because of the small sample size. Mann–Whitney test was used to identify differences between two independent groups of continuous variables. Wilcoxon Signed Ranks Test was used to compare paired measurements (within group comparisons). Significance was set at $p < 0.05$. Statistical analysis was performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

In total, 20 patients (three men, 17 women) with NAFLD (nine with NAFL and 11 with borderline or definite NASH) have to date completed the 8-week follow-up. Ten patients were randomly assigned to group 1 and 10 to group 2. Data of both groups at baseline and 8 weeks after the intervention are presented in table 1. At baseline, the two groups were matched for all clinical or biochemical parameters, including age, NAS, BMI, waist, systolic blood pressure (SBP), diastolic blood pressure (DBP), AST, ALT, GGT, cholesterol, triglycerides, HDL, LDL, potassium, sodium, glucose, insulin, HOMA-IR and QUICKI (table 1).

Comparisons between groups at week 8 revealed a borderline significant difference only in SBP ($p = 0.049$). Within-groups comparisons (baseline to week 8) revealed a significant difference in SBP in group 1 ($p = 0.011$). Within group 2, SBP was significantly decreased ($p = 0.034$) and potassium significantly increased ($p = 0.028$). Moreover, insulin was decreased within group 2 ($p = 0.013$). Although glucose was not decreased, HOMA-IR reached a significant decrease ($p = 0.047$). QUICKI was increased, though not significantly ($p = 0.074$).

A subgroup analysis, separately for NAFL and NASH subgroups, was consequently performed. No difference was found between NAFL subgroups comparisons at 8 weeks. Comparisons between NASH subgroups revealed significant differences in SBP ($p = 0.006$) and DBP ($p = 0.042$) (lower in group 2).

Table 1. Baseline and 8-week characteristics of both groups

	Group 1 (Vitamin E)		Group 2 (Spironolactone + Vitamin E)	
	Baseline	8 week	Baseline	8 week
Patients (number)	10	10	10	10
NAS	2.3 ± 0.4	–	3.6 ± 0.6	–
Age (years)	52.2 ± 5.2	–	57.4 ± 1.7	–
SBP (mmHg)	145 ± 4	132 ± 5 *	163 ± 7	142 ± 4 *ǂ
DBP (mmHg)	93 ± 4	88 ± 5	97 ± 3	91 ± 2
BMI (kg/m^2)	35.7 ± 1.7	34.1 ± 1.9	34.2 ± 1.6	32.9 ± 1.7
Waist (cm)	113 ± 4	113 ± 4	108 ± 3	105 ± 3
AST (U/L)	46 ± 15	36 ± 9	36 ± 5	29 ± 2
ALT (U/L)	55 ± 16	44 ± 12	43 ± 5	40 ± 5
AST/ALT ratio	0.86 ± 0.08	0.90 ± 0.08	0.87 ± 0.07	0.82 ± 0.10
GGT (U/L)	49 ± 9	39 ± 6	55 ± 15	42 ± 9
Cholesterol (mg/dl)	208 ± 8	225 ± 7	217 ± 13	225 ± 17
Triglycerides (mg/dl)	184 ± 28	192 ± 37	202 ± 32	212 ± 26
HDL (mg/dl)	50 ± 3	50 ± 3	49 ± 3	50 ± 4
LDL (mg/dl)	121 ± 11	137 ± 5	127 ± 9	133 ± 16
Potassium (mEq/l)	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.8 ± 0.1 *
Sodium (mEq/l)	143 ± 1	142 ± 1	141 ± 1	141 ± 1
Glucose (mg/dl)	108 ± 10	100 ± 8	110 ± 6	106 ± 7
Insulin (μU/ml)	13.8 ± 2.9	13.3 ± 2.7	15.3 ± 2.7	10.3 ± 1.6 *
HOMA-IR	3.4 ± 0.6	3.1 ± 0.5	4.4 ± 0.9	2.8 ± 0.5 *
QUICKI	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.34 ± 0.01

Data are presented as mean ± standard error of the mean (SE) or numbers
* $p < 0.05$ compared with baseline (within-groups comparison, Wilcoxon signed ranks test)
ǂ $p < 0.05$ compared with group 1 (between-groups comparison, Mann–Whitney test)
ALT, alanine transaminase; SBP, systolic blood pressure; AST, serum aspartate transaminase; DBP, diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment – insulin resistance; LDL, low-density lipoprotein; NAS, NAFLD Activity Score; QUICKI, Quantitative Insulin Sensitivity Check Index

No significant difference in any parameter was found within NAFL subgroups of either group 1 or group 2. Likewise, no significant difference in any parameter was found within NASH subgroup of group 1. Within NASH subgroup of group 2 (n = 7), apart from a significant decrease in SBP (p = 0.042) and DBP (p = 0.026), AST (41 ± 5 at baseline vs. 29 ± 2 U/l at week 8, p = 0.046) and insulin (17.3 ± 2.7 vs. 10.9 ± 1.7 U/l, respectively, p = 0.018) were also significantly decreased. ALT (50 ± 5 vs. 42 ± 6 U/l, respectively), GGT (69 ± 18 vs. 51 ± 11 U/l, respectively) and HOMA-IR (5.1 ± 1.0 vs. 3.1 ± 0.6, respectively) were also decreased, and QUICKI (0.31 ± 0.01 vs. 0.33 ± 0.01, respectively) was increased in the same subgroup, but their changes did not reach statistical significance.

Discussion

In this study, we found that the combined low-dose spironolactone and vitamin E therapeutic regimen had a favourable effect on serum insulin and HOMA-IR in NAFLD, possibly attributable to spironolactone action.

Patients with cirrhosis and heart failure complicated by T2DM appear to share the pathophysiology of decreased effective arterial blood volume due to splanchnic vasodilatation in cirrhosis and decreased cardiac output in heart failure, with consequential stimulation of the RAAS. Hyperaldosteronism has increasingly been recognised as a risk factor for myocardial and vascular fibrosis, and the low-dose aldosterone antagonists (25–50 mg/day) which are being used in cardiac patients are not natriuretic, thereby the mortality benefit relates primarily to their effect on cardiac and vascular fibrosis.[12] Aldosterone increases collagen accumulation in the fibroblasts and myocytes of the cardiac tissue, resulting in fibrosis and cardiac hypertrophy, whereas spironolactone attenuates or prevents formation of myocardial fibrosis.[18] Apart from its favourable effect on cardiac function, spironolactone may also provide therapeutic neuroprotective effects in the ischaemic brain after stroke by promoting neurogenesis and angiogenesis. Spironolactone reduces reactive oxygen metabolites (ROMs) production and apoptosis in the ischaemic striatum.[19,20] In addition, experimental evidence indicates that spironolactone seems to ameliorate hepatic fibrosis presumably via the inhibition of hepatic stellate cells activation.[21]

In view of the aforementioned data, we decided to introduce a sub-haemodynamic dose of spironolactone (25 mg/day) plus vitamin E used as an antioxidant in NAFLD; to our knowledge, this is the first report for the effect of spironolactone on serum biochemical parameters and IR in patients with NAFLD.

In addition to its crucial role in maintaining salt–water balance and blood pressure, the RAAS seems to be implicated in the development of IR and IR syndrome.[5,10,11] In vitro studies have shown that aldosterone induces IR in adipocytes and hepatocytes through ROMs-mediated degradation of insulin receptor substrate (IRS)-1 and IRS-2 and increasing mRNA expression of gluconeogenic enzymes.[22,23] Experimental studies have shown that blockade of mineralocorticoid receptor can improve IR in vivo.[24,25] Wada *et al*. showed for first time that spironolactone can improve hepatic steatosis, presumably by ameliorating IR and hepatic inflammation.[13] Furthermore, Noguchi *et al*. also reported that eplerenone, a selective aldosterone receptor antagonist, attenuated the progression of liver fibrosis in a rat model with NASH, possibly through the suppression of the activated hepatic stellate cells and neovascularisation.[26]

Interestingly, increased serum insulin and HOMA-IR and decreased QUICKI were shown in patients with primary aldosteronism compared with normotensive patients, which were rapidly restored to normal after either unilateral adrenalectomy or administration of spironolactone (50–300 mg/day).[27] Furthermore, it has been recently reported that normolipidaemic, non-diabetic, non-obese patients with primary aldosteronism are more insulin resistant and have higher prevalence of NAFLD than normotensive controls, indicating greater risk for IR and NAFLD in these patients.[28] Although there is no direct evidence as to whether NAFLD patients have higher serum aldosterone compared with matched controls, this speculation may be correct regarding the effect of spironolactone on insulin and HOMA-IR in this study.

The favourable effect of spironolactone on insulin and HOMA-IR in patients with NAFLD was similar to that recently mentioned by Wada *et al*. in a mouse model with diet-induced diabetes and NAFLD.[13] However, the effect of spironolactone on serum glucose and lipids was not similar to that described by Wada *et al*. Different doses or routes of administration would partly account for differences in serum glucose and lipids between the work of Wada *et al*. and our study. Wada *et al*. gave 16 mg/kg of spironolactone daily via a subcutaneous implant route in mice,[13] which is a relatively high dose when used as a clinical therapy in humans. Furthermore an 8-week period may be sufficient in a mouse model, but not in humans, for final conclusions. In any case, experimental results cannot be directly applicable to humans, and should always be carefully interpreted in human terms.

There are limited data for the impact of spironolactone on serum insulin, glucose and lipids in patients with IR syndrome. Spironolactone has been mainly investigated in women with polycystic ovary syndrome (PCOS), which is considered to be the ovarian component of IR syndrome, because of its anti-androgenic action. In a recent 3-month RCT, spironolactone (100 mg/day) plus ethinyl estradiol/cyproterone acetate was found to significantly decrease both insulin and HOMA-IR.[29] Similarly, a 12-month spironolactone (100 mg/day) administration significantly decreased insulin and HOMA-IR in overweight women with PCOS.[30]

The effect of spironolactone on serum glucose and lipids in women with PCOS is controversial. In one of the above-mentioned studies,[29] spironolactone plus ethinyl

estradiol/cyproterone significantly decreased serum LDL and total cholesterol, but had no effect on HDL and triglycerides. In the other study,[30] spironolactone had no effect on serum lipids and glucose, apart from a decrease in triglycerides. In another 3-month study, spironolactone (100 mg/day) had no effect on serum glucose and lipids in patients with PCOS.[31] Similarly, spironolactone did not significantly change serum glucose and lipids in patients with essential hypertension.[32] Differences in study population, duration and/or drug co-administration might account for these differing results.

Spironolactone in patients with T2DM has been investigated mainly for its favourable impact on diabetic nephropathy.[33,34] However, data for the metabolic effect of spironolactone in patients with T2DM are limited. In a 3-month RCT, spironolactone (50 mg/day) had no effect on serum glucose, cholesterol, triglycerides, insulin, and HOMA-IR in patients with T2DM complicated with diabetic nephropathy,[35] but diabetic nephropathy may interfere with serum insulin, unlike in uncomplicated patients with NAFLD or PCOS.

A trend of improvement in liver function test was observed within NASH subgroup of group 2. To our knowledge, there is no study investigating the effect of spironolactone on serum transaminases in patients with IR syndrome. In rat models, spironolactone decreased ALT and liver toxicity induced by dimethyl mercury[36] or D-galactosamine.[37]

In this study, HOMA-IR was significantly decreased within group 2, whereas QUICKI showed a trend towards increase (meaning IR improvement) in the same group without reaching the level of statistical significance. This is not unexpected, since it has been previously reported that both HOMA and QUICKI reflect insulin action expressed by clamps,[16,17] but they do not offer the same information about IR, thereby expressing different aspects of insulin action.[38]

Our series study has certain limitations. First, the effect of IR was not the primary aim of this RCT. Second, the sample size, despite being well matched, was small. Third, the hyperinsulinaemic–euglycaemic clamp technique, which is the gold standard for evaluation of IR, was not performed in our patients. However, HOMA-IR and QUICKI have acceptable correlation with hyperinsulinaemic–euglycaemic clamp and are widely used.[16,17] Furthermore, we did not measure aldosterone levels and plasma renin activity, as this was beyond the aim of this interim analysis. Finally, our patients did not undergo a repeat biopsy to investigate the effect of spironolactone in liver histology; however an 8-week period is too short in human terms for effects to be seen, thereby raising ethical considerations.

Conclusion

A favourable effect of spironolactone and vitamin E on serum insulin and HOMA-IR in patients with NAFLD has been shown, possibly attributed mainly to spironolactone action. Given that this is a preliminary report, a large-scale human trial is needed to clarify whether spironolactone could be a beneficial therapeutic approach for IR syndrome, including NAFLD. If it was confirmed that spironolactone has anti-inflammatory and anti-fibrogenic effects on the liver, similar to the heart and the above-mentioned experimental model with diabetes and NAFLD, it would become an inexpensive therapeutic approach for the management of NAFLD patients.

Clinical trial declaration

ClinicalTrials.gov NCT01147523

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Conflict of interest

No author declared any conflict of interest related to this manuscript.

References

1. Bellentani S and Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; 8(Suppl 1): 4–8.
2. Ahmed MH, Abu EO and Byrne CD. Non-Alcoholic Fatty Liver Disease (NAFLD): New challenge for general practitioners and important burden for health authorities? *Prim Care Diabetes* 2010; 4: 129–137.
3. Polyzos SA, Kountouras J and Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009; 72: 299–314.
4. Lewis JR and Mohanty SR. Nonalcoholic fatty liver disease: a review and update. *Dig Dis Sci* 2010; 55: 560–578.
5. Polyzos SA, Kountouras J, Zavos C and Tsiaousi E. The role of adiponectin in the pathogenesis and treatment of nonalcoholic fatty liver disease. *Diabetes Obes Metab* 2010; 12: 365–383.
6. Musso G, Gambino R, Cassader M and Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010; 52: 79–104.
7. Sanyal AJ, Chalasani N, Kowdley KV, *et al*. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; 362: 1675–1685.
8. Athyros VG, Mikhailidis DP, Didangelos TP, *et al*. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study. *Curr Med Res Opin* 2006; 22: 873–883.
9. Filippatos TD and Elisaf MS. Combination drug treatment in patients with non-alcoholic fatty liver disease. *World J Hepatol* 2010; 2: 139–142.
10. Hirose A, Ono M, Saibara T, *et al*. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology* 2007; 45: 1375–1381.
11. Giacchetti G, Sechi LA, Rilli S and Carey RM. The renin–angiotensin–aldosterone system, glucose metabolism and diabetes. *Trends Endocrinol Metab* 2005; 16: 120–126.

12. Bansal S, Lindenfeld J and Schrier RW. Sodium retention in heart failure and cirrhosis: potential role of natriuretic doses of mineralocorticoid antagonist? *Circ Heart Fail* 2009; 2: 370–376.
13. Wada T, Kenmochi H, Miyashita Y, *et al*. Spironolactone improves glucose and lipid metabolism by ameliorating hepatic steatosis and inflammation and suppressing enhanced gluconeogenesis induced by high-fat and high-fructose diet. *Endocrinology* 2010; 151: 2040–2049.
14. Kleiner DE, Brunt EM, Van Natta M, *et al*. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41: 1313–1321.
15. Chalasani NP, Sanyal AJ, Kowdley KV, *et al*. Pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis: PIVENS trial design. *Contemp Clin Trials* 2009; 30: 88–96.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
17. Katz A, Nambi SS, Mather K, *et al*. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402–2410.
18. Struthers AD and MacDonald TM. Review of aldosterone- and angiotensin II-induced target organ damage and prevention. *Cardiovasc Res* 2004; 61: 663–670.
19. Oyamada N, Sone M, Miyashita K, *et al*. The role of mineralocorticoid receptor expression in brain remodeling after cerebral ischemia. *Endocrinology* 2008; 149: 3764–3777.
20. Dorrance AM. Stroke therapy: is spironolactone the Holy Grail? *Endocrinology* 2008; 149: 3761–3763.
21. Fujisawa G, Muto S, Okada K, Kusano E and Ishibashi S. Mineralocorticoid receptor antagonist spironolactone prevents pig serum-induced hepatic fibrosis in rats. *Transl Res* 2006; 148: 149–156.
22. Wada T, Ohshima S, Fujisawa E, Koya D, Tsuneki H and Sasaoka T. Aldosterone inhibits insulin-induced glucose uptake by degradation of insulin receptor substrate (IRS) 1 and IRS2 via a reactive oxygen species-mediated pathway in 3T3-L1 adipocytes. *Endocrinology* 2009; 150: 1662–1669.
23. Yamashita R, Kikuchi T, Mori Y, *et al*. Aldosterone stimulates gene expression of hepatic gluconeogenic enzymes through the glucocorticoid receptor in a manner independent of the protein kinase B cascade. *Endocr J* 2004; 51: 243–251.
24. Guo C, Ricchiuti V, Lian BQ, *et al*. Mineralocorticoid receptor blockade reverses obesity-related changes in expression of adiponectin, peroxisome proliferator-activated receptor-gamma, and proinflammatory adipokines. *Circulation* 2008; 117: 2253–2261.
25. Hirata A, Maeda N, Hiuge A, *et al*. Blockade of mineralocorticoid receptor reverses adipocyte dysfunction and insulin resistance in obese mice. *Cardiovasc Res* 2009; 84: 164–172.
26. Noguchi R, Yoshiji H, Ikenaka Y, *et al*. Selective aldosterone blocker ameliorates the progression of non-alcoholic steatohepatitis in rats. *Int J Mol Med* 2010; 26: 407–413.
27. Catena C, Lapenna R, Baroselli S, *et al*. Insulin sensitivity in patients with primary aldosteronism: a follow-up study. *J Clin Endocrinol Metab* 2006; 91: 3457–3463.
28. Fallo F, Dalla PA, Tecchio M, *et al*. Nonalcoholic fatty liver disease in primary aldosteronism: a pilot study. *Am J Hypertens* 2010; 23: 2–5.
29. Kebapcilar L, Taner CE, Kebapcilar AG, Alacacioglu A and Sari I. Comparison of four different treatment regimens on coagulation parameters, hormonal and metabolic changes in women with polycystic ovary syndrome. *Arch Gynecol Obstet* 2009; (E-pub ahead of print).
30. Zulian E, Sartorato P, Benedini S, *et al*. Spironolactone in the treatment of polycystic ovary syndrome: effects on clinical features, insulin sensitivity and lipid profile. *J Endocrinol Invest* 2005; 28: 49–53.
31. Nakhjavani M, Hamidi S, Esteghamati A, Abbasi M, Nosratian-Jahromi S and Pasalar P. Short term effects of spironolactone on blood lipid profile: a 3-month study on a cohort of young women with hirsutism. *Br J Clin Pharmacol* 2009; 68: 634–637.
32. Yutaka M, Mifune M, Kubota E, Itoh H and Saito I. Comparison of effects of low dose of spironolactone and a thiazide diuretic in patients with hypertension treated with an angiotensin-converting enzyme inhibitor or an angiotensin type 1 receptor blocker. *Clin Exp Hypertens* 2009; 31: 648–656.
33. Ustundag A, Tugrul A, Ustundag S, Sut N and Demirkan B. The effects of spironolactone on nephron function in patients with diabetic nephropathy. *Ren Fail* 2008; 30: 982–991.
34. Davidson MB, Wong A, Hamrahian AH, Stevens M and Siraj ES. Effect of spironolactone therapy on albuminuria in patients with type 2 diabetes treated with angiotensin-converting enzyme inhibitors. *Endocr Pract* 2008; 14: 985–992.
35. Matsumoto S, Takebayashi K and Aso Y. The effect of spironolactone on circulating adipocytokines in patients with type 2 diabetes mellitus complicated by diabetic nephropathy. *Metabolism* 2006; 55: 1645–1652.
36. Kourounakis PN and Rekka E. Effect of spironolactone on dimethyl mercury toxicity. A possible molecular mechanism. *Arzneimittelforschung* 1994; 44: 1150–1153.
37. Mihas AA, Foster MM, Barnes S, Mihas TA, Hirschowitz BI and Spenney JG. Modification of D-galactosamine-induced liver injury in the rat by spironolactone. *Pharmacology* 1979; 18: 235–240.
38. Skrha J, Haas T, Sindelka G, *et al*. Comparison of the insulin action parameters from hyperinsulinemic clamps with homeostasis model assessment and QUICKI indexes in subjects with different endocrine disorders. *J Clin Endocrinol Metab* 2004; 89: 135–141.