DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2000; **16**: 2–7.

RESEARCH ARTICLE

Treatment with metformin of non-diabetic men with hypertension, hypertriglyceridaemia and central fat distribution: the BIGPRO 1.2 trial

M. Aline Charles[1]*
Eveline Eschwège[1]
Pierre Grandmottet[2]
Françoise Isnard[3]
Jean-Marie Cohen[4,5]
Jean-Louis Bensoussan[5]
Hervé Berche[5]
Ouri Chapiro[5]
Philippe André[3]
Philippe Vague[6]
Irène Juhan-Vague[6]
Jean-Marie Bard[7]
Michel Safar[2]

[1]*National Institute of Health and Medical Research (INSERM), unit 258, Villejuif, France*
[2]*National Pedagogic Association for the Teaching of Therapeutics (APNET), France*
[3]*Laboratoires LIPHA, Suresnes (FI) and Lyon (PA), France*
[4]*Organize and Promote Epidemiological Networks (OPEN ROME), Paris, France*
[5]*Coordinators of the General Practitioners, Paris (JMC), Castelmaurou (JLB), Compiègne (HB), Corbeil-Essonnes (OC), France*
[6]*Hematology (IJ-V) and Diabetology (PV) Departments, La Timone Hospital, Marseille, France*
[7]*Biochemistry Laboratory, University of Pharmacy, Nantes, France*

*Correspondence to: INSERM U 258, 16 avenue Paul Vaillant-Couturier, F-94807 Villejuif, France.
E-mail: charles@vjf.inserm.fr

Received: 18 August 1999
Revised: 20 October 1999
Accepted: 2 November 1999
Published online: 17 November 1999

Abstract

Background In the BIGPRO 1 trial, one year of treatment with metformin in non-diabetic obese subjects with a central fat distribution had no significant effect on fasting plasma triglyceride concentration or on blood pressure despite a decrease in weight, fasting plasma insulin and glucose concentrations. To re-evaluate the effect of metformin on fasting triglyceride concentration and on blood pressure, the BIGPRO 1.2 trial included non-diabetic men ($n=168$) with a fasting plasma triglyceride concentration ≥ 1.7 and ≤ 6.5 mmol/l, high blood pressure (systolic ≥ 140 and ≤ 180 and/or diastolic ≥ 90 and ≤ 105 mmHg, or treatment for hypertension) and a waist-to-hip ratio ≥ 0.95.

Methods A randomised double-blind trial comparing metformin treatment (850 mg bid) with placebo.

Results Metformin had no significant effect either on blood pressure or plasma triglyceride concentration. In comparison with the placebo group, fasting plasma insulin ($p<0.04$), total cholesterol ($p<0.05$) and Apo B ($p<0.008$) concentrations decreased more in the metformin group in the BIGPRO 1.2 trial, confirming most of the previous results of the BIGPRO 1 trial. Tissue plasminogen activator antigen concentration decreased significantly ($p<0.01$) only in the metformin group, but this was not significantly different from the placebo group ($p<0.12$); further, there were no significant differences in the change in plasminogen activator inhibitor 1.

Conclusions The consistency of the two BIGPRO trials supports the conclusion that metformin affects several cardiovascular risk factors favourably in non-diabetic subjects with a central fat distribution.

Keywords metformin; waist-to-hip ratio; triglyceride; hypertension; central obesity

Introduction

Upper body fat distribution is a risk factor for cardiovascular morbidity and mortality, independent of classical cardiovascular risk factors [1–3]. Subjects with this type of fat distribution often present with decreased insulin sensitivity along with glucose intolerance, hypertriglyceridaemia and a low HDL-cholesterol concentration, and mild hypertension [4]. This constellation of abnormalities, known as the insulin resistance or metabolic syndrome, is thought to be responsible for the increased cardiovascular risk [5]. The BIGPRO 1 trial [6], in 324 obese non-diabetic men and women

with a high waist-to-hip ratio, was initiated to test whether a one-year treatment with metformin would reduce insulin resistance (as measured by fasting plasma insulin concentrations) and the other features of the insulin resistance syndrome. Metformin decreased the fasting plasma insulin and glucose concentrations, and encouraged maintenance of weight loss [6]. However, these effects did not translate into a significant change either in blood pressure, plasma triglyceride or HDL-cholesterol concentrations, in comparison with the placebo group. One hypothesis was that the subjects included in the BIGPRO 1 trial, two-thirds of whom were women, were too normal in terms of their initial blood pressure and plasma triglyceride concentrations. The aim of BIGPRO 1.2 was to re-evaluate in non-diabetic subjects the effect of metformin on plasma fasting triglyceride concentration, the main end-point, and on blood pressure. The population selected to answer this question was men with a high waist-to-hip ratio, a high fasting plasma triglyceride concentration and treated or untreated mild hypertension. The study was designed as a randomised, double-blind, placebo controlled trial in parallel groups. A second aim was to confirm the effect of metformin observed in BIGPRO 1, on cardiovascular risk factors that are not classically included in the insulin resistance syndrome, namely total and LDL plasma cholesterol [6], and two plasma markers of endothelial damage, the tissue plasminogen activator antigen (t-PA-ag) and the von Willebrand factor (vWF) [7].

Subjects and methods

Patients and study protocol

The study investigators were 105 general practitioners from throughout France. They recruited men aged between 18 and 55 years without diabetes, patent cardiovascular disease, known renal or hepatic insufficiency, mental illness or any life-threatening condition. Height and weight (measured in underwear without shoes) were recorded along with waist and hip circumferences, measured respectively at the mid-distance between the lower rib margin and the iliac crest, and at the level of the buttock which yielded the greatest circumference. Blood pressure was recorded twice at a one minute interval with a large cuff on subjects resting in the supine position, and the average was used.

Men were included in the study if they agreed to participate in a therapeutic trial and had both:

- systolic blood pressure ≥ 140 and ≤ 180 mmHg and/or diastolic blood pressure ≥ 90 and ≤ 105 mmHg, and/or treatment for hypertension
- a waist-to-hip circumference ratio 0.95 with a body mass index (BMI) [weight (kg)/height^2 (cm)] < 33 kg/m^2.

Inclusion in the trial was confirmed between 10 and 60 days later, if:

- the above clinical criteria were met again
- the fasting plasma triglyceride concentration was ≥ 1.7 and ≤ 6.8 mmol/l (150 and 600 mg/dl)
- the plasma creatinine concentration was < 130 μmol/l, the SGOT and SGPT concentrations were lower than four times the upper limit of normal, and the fasting blood glucose concentration was < 7.8 mmol/l.

Subjects taking lipid-lowering drugs could be included if they had a fasting plasma triglyceride concentration in the required range after at least one month of treatment withdrawal.

The men were randomised to receive oral metformin (850 mg twice daily) or placebo for three months following a double blind procedure. They were not given any dietary counselling. Treatment by antidiabetic or lipid-lowering drugs was not permitted during the trial and it was recommended not to change other medications. Clinical examinations were performed one and a half, and three months after inclusion, with assessments of treatment compliance and side-effects.

Biological evaluations

Blood samples were taken just after the first visit with the general practitioner (pre-inclusion) and 10 days before the end of the three-month treatment period. Venous blood was drawn with minimal stasis from fasting subjects in a local laboratory. Plasma and serum samples were stored at -30°C until collection by the central laboratory (CERBA, Cergy Pontoise, France) for measurements of:

- plasma glucose, creatinine, uric acid, SGOT, SGPT, GGT, total cholesterol, HDL-cholesterol (after selective precipitation of VLDL and LDL) and triglyceride concentrations by enzymatic methods adapted to an automatic analyser (Hitachi 911, Boehringer Manheim)
- serum Apo AI and Apo B by immunonephelemetry (Behring).

At the final biological evaluation, the above parameters were measured, excepting creatinine, SGPT and SGOT.

In addition, plasma insulin (by radioimmunoassay at CERBA) and haemostatic parameters were measured after the end of the study, using frozen samples obtained at the inclusion and final evaluations. Haemostatic parameters were assayed at the Haematology Department of La Timone Hospital in Marseille: PAI-1 activity (PAI-1-act) using a commercially available kit (Biopool, Umea, Sweden); PAI-1 antigen (PAI-1-ag), t-PA-ag and vWF by ELISA with kits from Diagnostica Stago, Asnières, France; fibrinogen was estimated by a chronometric method. Plasma for measurements of haemostatic parameters was available for 139 subjects at inclusion and for 113 subjects at both the inclusion and final evaluations.

Statistical analyses

Results for the variables that had a skewed distribution (BMI, concentrations of plasma insulin, triglyceride and of the haemostatic parameters) were expressed as geometric means and 95% confidence intervals. Placebo and metformin groups were compared at baseline by Student's *t*-test and χ^2 test. The treatment effect was evaluated by comparing, between the metformin and placebo groups, the changes between the inclusion and three-month visits, with paired *t*-tests, on an intent-to-treat-basis. There were three values of the changes in fasting insulin concentration that exceeded the mean ± 3 SD, which were re-coded to the next highest value of the distribution. We estimated that a sample size of 85 subjects per group was adequate to enable demonstration of a difference in the average change in plasma triglyceride concentration of at least 0.34 mmol/l (30 mg/dl) with an α risk of 0.05 and a power of 80%. All analyses were performed with the SAS package (SAS Institute Inc., Cary, NC, USA).

Results

The inclusion period lasted one and a half years. Among the 340 subjects included in the trial, only 168 could be randomised to the metformin or placebo treatments. The main reasons for non inclusion were: 20% of the subjects did not return for the inclusion visit, 64% had a fasting plasma triglyceride concentration that was too low and in 10% it was too high. One subject had a fasting blood glucose concentration greater than 7.8 mmol/l. Among the 85 subjects assigned to the placebo group and the 83 subjects assigned to the metformin group, clinical data were missing at the final evaluation in six (7%) and in two (2%) subjects respectively (ns). Corresponding figures for the biological evaluation were four (5%) and five (6%), (ns). Three subjects (4%) in the placebo group and eight (10%) in the metformin group stopped the treatment before the end of the trial (because of side-effects in five of the metformin-treated subjects and in none of the placebo-treated subjects). Five (6%) in the placebo group and 30 subjects (36%) in the metformin group ($p<0.001$) complained of gastro-intestinal side-effects, mainly diarrhoea. Other adverse side effects were reported by 10.5% of the placebo and 7.0% of the metformin treated subjects (ns) but no specific effect was described by more than two subjects.

Men included in the trial had a mean fasting plasma triglyceride concentration of 2.77 and 2.83 mmol/l in the metformin and placebo groups, respectively (ns) (Table 1). Of the subjects 37% (metformin: 42%, placebo 34%) were treated for hypertension, mainly with β-blockers (45%) and ACE inhibitors (22%). The men included in the two treatment groups were comparable except for the GGT and the vWF concentrations, which were higher in the metformin group ($p<0.06$ and $p<0.04$, respectively) (Table 1).

Table 1. Clinical features of the men included in the metformin and placebo groups at baseline

	Metformin	Placebo
Number	83	85
Age (y) (median, range)	45, 20–56	46, 26–55
BMI (kg/m^2)	28 (24–33)	29 (24–35)
WHR	1.00 $\pm$ 0.03	1.01 $\pm$ 0.04
Systolic blood pressure (mmHg)	144 $\pm$ 11	144 $\pm$ 13
Diastolic blood pressure (mmHg)	91 $\pm$ 8	91 $\pm$ 10
% with antihypertensive treatment	42%	34%
SGPT (U/l)	50 $\pm$ 31	46 $\pm$ 22
SGOT (U/l)	31 $\pm$ 13	28 $\pm$ 9
GGT (U/l)	76 $\pm$ 57	61 $\pm$ 41
Fasting blood glucose (mmol/l)	5.60 $\pm$ 0.74	5.55 $\pm$ 0.79
Fasting insulin (pmol/l)	61 (20–190)	62 (20–186)
Total cholesterol (mmol/l)	6.68 $\pm$ 1.31	6.56 $\pm$ 1.26
HDL-cholesterol (mmol/l)	1.06 $\pm$ 0.34	1.09 $\pm$ 0.46
Apo AI (g/l)	1.55 $\pm$ 0.23	1.47 $\pm$ 0.34
Apo B (g/l)	1.53 $\pm$ 0.38	49 $\pm$ 0.36
Triglyceride (mmol/l)	2.77 (1.48–5.17)	2.83 (1.52–5.28)
PAI1 activity (IU/ml)[a]	24 (6–100)	24 (4–157)
PAI1-ag (ng/ml)[a]	75 (17–328)	78 (20–314)
t-PA-ag (ng/ml)[a]	12 (7–22)	12 (7–23)
vWF (U/l)[a]	1.2 (0.5–2.5)	1.0 (0.4–2.5)
Fibrinogen (g/l)[a]	3.2 (2.1–4.9)	3.1 (1.9–5.0)

Quantitative variables expressed as arithmetic mean $\pm$ SD, or geometric mean (95% confidence interval).
[a]Results for 69 and 70 subjects in the metformin and placebo groups, respectively.

There were significant differences between the two treatment groups for changes in fasting insulin ($p<0.04$), total cholesterol ($p<0.05$), Apo B ($p<0.008$) and GGT ($p<0.02$) concentrations, which all decreased in the metformin group but did not change in the placebo group (Table 2). There was also a trend for a greater decrease in weight ($p<0.14$) and fasting glucose ($p<0.15$) (Table 2). Plasma triglyceride concentration fell both in the metformin and in the placebo groups, by an average

Table 2. Comparison of the changes (three months – entry) in clinical and biological variables between the metformin and the placebo groups

	Metformin	Placebo	*p*-value
Number	83	85	
Weight (kg)	-0.5 ± 2.8	0.1 ± 2.1	0.14
WHR	-0.008 ± 0.034	-0.015 ± 0.032	0.16
Systolic blood pressure (mmHg)	-5 ± 11	-2 ± 12	0.18
Diastolic blood pressure (mmHg)	-4 ± 9	-2 ± 9	0.26
GGT (U/l)	-12 ± 25	0 ± 28	0.02
Fasting blood glucose (mmol/l)	-0.16 ± 0.83	0.02 ± 0.66	0.15
Fasting insulin (pmol/l)	-10 ± 38	0 ± 29	0.04
Total cholesterol (mmol/l)	-0.34 ± 0.94	-0.02 ± 1.10	0.05
HDL-cholesterol (mmol/l)	-0.06 ± 0.31	-0.12 ± 0.42	0.33
Apo AI (g/l)	-0.01 ± 0.21	0.03 ± 0.31	0.31
Apo B (g/l)	-0.09 ± 0.22	0.02 ± 0.30	0.008
Triglyceride (mmol/l)	-0.23 ± 1.05	-0.11 ± 1.20	0.51
PAI1 activity (IU/ml)[a]	-4 ± 15	-5 ± 18	0.85
PAI1-ag (ng/ml)[a]	-21 ± 64	-16 ± 62	0.72
t-PA-ag (ng/ml)[a]	-1.2 ± 3.0	-0.4 ± 2.5	0.12
vWF (U/l)[a]	-0.06 ± 0.42	0.02 ± 0.46	0.40
Fibrinogen (g/l)[a]	-0.09 ± 0.82	0.06 ± 0.80	0.39

Mean $\pm$ SD.
[a]Results for 55 and 58 subjects in the metformin and placebo groups, respectively.

of 0.23 and 0.11 mmol/l respectively (ns) (Table 2). The results on lipid changes were unaffected by excluding men treated by β-adrenoceptor blockers. The PAI1 activity and antigen decreased significantly ($p < 0.05$) during the trial, equally in the metformin and placebo groups. In contrast, t-PA-ag decreased significantly in the metformin group ($p < 0.01$), but the mean changes did not differ significantly between the metformin and placebo groups ($p < 0.12$).

Discussion

The BIGPRO 1.2 study was designed specifically to test for an effect of metformin on fasting plasma triglyceride concentration: it had 80% power to detect a change of at least 0.34 mmol/l. However, in these middle-aged men with a central fat distribution, hypertension and high fasting plasma triglyceride concentration, the reduction in triglyceride concentration that was observed after three months of treatment with metformin was not significant compared with that observed in the placebo group. The difference between the changes in the two groups was only 0.12 mmol/l, about one-third of the 0.34 mmol/l difference that had been considered to be of clinical importance in these hypertriglyceridaemic subjects and used for the calculation of the number of subjects required for the trial. This non-significant result confirms several other negative randomised clinical trials, in non-diabetic hypertensive [8,9], dyslipidaemic [10,11] or centrally obese [BIGPRO1: 6] subjects, and in subjects with Type 2 diabetes (comparing metformin with either placebo [12]: protocol 1, [13–15], guar [16], sulphonylureas [17–21] or insulin [22–24]). There is no obvious explanation why some studies concluded that metformin had an effect on plasma triglyceride concentrations [25], 12: protocol 2, [26–29]; in terms of the baseline characteristics of the subjects, quality of glycaemic control, weight loss, duration of the study, dose of metformin or study power. However, particular attention has to be given to the largest of these clinical trials [12]: protocol 2], which compared the effect of metformin to that of glyburide and of a combination of the two drugs. In this trial, 632 patients with type 2 diabetes with an initial mean fasting plasma triglyceride concentration of 2.5 mmol/l (220 mg/dl) were included. There was a significant fall in triglyceride concentration (of on average 0.20 mmol/l, 18 mg/dl) in the metformin and in the combination groups compared to the glyburide group, despite similar glycaemic control. Most of the other studies did not have the power to detect such a small effect. Therefore, metformin may have a modest effect on fasting plasma triglyceride concentration in hypertriglyceridaemic patients with Type 2 diabetes. In non-diabetic hypertriglyceridaemic subjects, only a large size effect of metformin can be excluded.

All the studies discussed above considered only fasting plasma triglyceride concentrations. In Type 2 diabetic and glucose intolerant subjects [30–32], three months of treatment with metformin has been shown to greatly affect postprandial lipaemia Whether this effect of metformin exists in subjects with normal glucose tolerance is not known but deserves further attention, given the potential impact of postprandial lipaemia on cardiovascular risk [33,34].

Because hypertriglyceridaemia is the main lipid abnormality in Type 2 diabetic, obese or hypertensive subjects, most of the attention about the effect of metformin on plasma lipid concentration has been focused on triglyceride. However, in contrast to the mostly negative results for triglyceride, there is evidence that metformin is able to decrease plasma total and LDL cholesterol, and Apo B concentrations in Type 2 diabetic as well as in non-diabetic subjects. In addition to the BIGPRO 1 [6] and BIGPRO 1.2 studies, all [9–11,25] but one [8] of the randomised clinical trials in non-diabetic subjects and 10 [12]: protocol 1 and [2,15,16,22,24,26–29] of the randomised trials in Type 2 diabetic patients, including the two largest studies [12] have reached this conclusion. The size of the effect is modest, between 3 and 10% in most studies, but the mean baseline total cholesterol concentrations were either normal or only moderately elevated.

The second main end-point studied in the BIGPRO 1.2 trial, the change in blood pressure with metformin treatment, did not differ significantly from that observed in the placebo group. This result is again concordant with BIGPRO 1 and is in agreement with most published randomised clinical trials in diabetic [12,14,19,22,26,29] as well as in non-diabetic [8,9,35,36] subjects except in three studies [24,25,28].

In the BIGPRO 1.2 trial, there was a significant decrease in fasting insulin and in GGT concentration with metformin treatment. The effect on fasting insulin is a well known action of the drug which had been documented both in diabetic and non-diabetic subjects and is interpreted as resulting from an improvement in insulin sensitivity [37]. In BIGPRO 1.2, this action was observed in the metformin group in the absence of significant weight loss. The effect on GGT was unexpected. As GGT concentrations were higher in the metformin group at baseline ($p < 0.06$), a regression to the mean effect cannot be excluded. However if confirmed, this effect may be of interest in view of the strong predictive effect of GGT concentration for conversion to Type 2 diabetes [38].

The most significant effects of metformin in BIGPRO 1 were the reductions in two markers of endothelial damage, the t-PA-ag and vWF concentrations, which led to the hypothesis that metformin could have a beneficial effect at the endothelial level [7]. There was a similar decline in t-PA-ag concentration after three months of treatment with metformin in BIGPRO 1.2 (-1.2 ± 3.0 ng/ml, Table 2) as that observed after one year in BIGPRO 1 (-1.1 ± 3.1 ng/ml) [6]. However, because of a greater decline in the placebo group in BIGPRO 1.2 (-0.3 vs -0.2 ng/ml), the change in BIGPRO 1.2 was not significant ($p < 0.12$). The situation is quite different for

the vWF for which a slow steady decrease was observed in BIGPRO 1 [7]. The change in comparison with the placebo group reached statistical significance only after one year of treatment, but was not significant at the six month evaluation. Therefore, it is not surprising to see only a slight and insignificant reduction in the vWF concentration in the metformin group at three months in the present study. As for the fibrinolysis parameters, PAI1 activity and PAI1 antigen, neither BIGPRO 1 [7] nor BIGPRO 1.2 demonstrated a significant action of metformin. Weight loss was at least partly responsible for the decline in PAI1 observed both in the placebo and the metformin groups in BIGPRO 1 [7]. In BIGPRO 1.2, a more modest but still significant decrease in PAI1 was observed in the placebo group, despite an average weight rise of 0.1 kg.

The consistency of the metformin effect in two BIGPRO studies permits us to conclude that in non-diabetic subjects with a central fat distribution, treatment with metformin can be expected to improve insulin sensitivity and decrease blood glucose concentrations in glucose intolerant subjects (shown in BIGPRO 1), to decrease total and LDL-cholesterol concentration and two markers of endothelial damage. In addition, if dietary recommendations are associated, as was the case in BIGPRO 1, metformin favours the maintenance of weight loss and of its consequences, namely a further increase in insulin sensitivity and improved fibrinolysis [6,7]. A fall in blood pressure and in plasma triglyceride concentrations may occur as a consequence of the weight loss.

Acknowledgements

We gratefully acknowledge the contributions of all the general practitioner investigators in the study, of Kathy Bean for the coordination of the data collection, of Nadine Thibult for statistical analysis, and of Drs Jacques Amar, Olivier Chassany, Vincent Durlach, Jean-Michel Guliana, Michel Slama members of the group advising the data collection committee. The study was supported by funds from the LIPHA Pharmaceutical Company.

References

1. Larsson B, Svärdsudd K, Welin L, Björntorp P, Tibbling G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: a 12 year follow-up of participants in the study of men born in 1913. *Br Med J* 1984; **288**: 1401–1404.
2. Ducimetière P, Richard JL, Cambien F. The pattern of subcutaneous fat distribution in middle-aged men and the risk of coronary heart disease: the Paris Prospective Study. *Int J Obes* 1986; **10**: 229–240.
3. Kannel WB, Cupples LA, Ramaswami R, Strokes III J, Kreger BE, Higgins M. Regional obesity and the risk of cardiovascular disease: the Framingham study. *J Clin Epidemiol* 1991; **44**: 183–190.
4. Björntorp P. 'Portal' adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* 1990; **10**: 493–496.
5. Alberti KGMM, Zimmet PZ for the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539–553.
6. Fontbonne A, Charles MA, Juhan-Vague I, *et al*. The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution. Results of the BIGPRO 1 trial. *Diabetes Care* 1996; **19**: 920–926.
7. Charles MA, Morange P, Eschwège E, André P, Vague P, Juhan-Vague I, on behalf of the BIGPRO Study Group. The effect of weight change and of metformin on fibrinolysis and von Willebrand factor in obese non diabetic subjects. The BIGPRO 1 Study. *Diabetes Care* 1998; **21**: 1967–1972.
8. Dorella M, Giusto M, Da Tos V, *et al*. Improvement of insulin sensitivity by metformin treatment does not lower blood pressure of nonobese insulin-resistant hypertensive patients with normal glucose tolerance. *J Clin Endocrinol Metab* 1996; **81**: 1568–1574.
9. Herlitz H, Landin K, Widgren B. Relationship between sodium–lithium contertransport and insulin sensitivity in mild hypertension. *J Int Hypertension* 1996; **239**: 235–240.
10. Carlsen SM, Rossvoll O, Bjerve KS, Følling I. Metformin improves blood lipid pattern in nondiabetic patients with coronary heart disease. *J Intern Med* 1996; **239**: 227–231.
11. Pentikäinen PJ, Voutilainen E, Aro A, Uusitupa M, Penttilä I, Vapaatalo H. Cholesterol lowering effect of metformin in combined hyperlipidemia: placebo controlled double blind trial. *Ann Med* 1990; **22**: 307–312.
12. De Fronzo R, Goodman A, the Multicenter Metformin Study Group. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995; **333**: 541–549.
13. Johnson AB, Webster JM, Sum C-F, *et al*. The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight Type II diabetic patients. *Metabolism* 1993; **42**: 1217–1222.
14. Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS. The effects of metformin on glycaemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. *Diabetes Care* 1998; **21**: 701–705.
15. Lalor BC, Bhatnagar D, Winocour PH, *et al*. Placebo-controlled trial of the effects of guar gum and metformin on fasting blood glucose and serum lipids in obese Type 2 diabetic patients. *Diabet Med* 1990; **7**: 242–245.
16. Wilson JA, Scott MM, Gray RS. A comparison of metformin versus guar in combination with sulfonylureas in the treatment of non insulin dependent diabetes. *Horm Metab Res* 1989; **21**: 317–319.
17. Sundaresan P, Lykos D, Dahler A, Diamond T, Morris R, Howes LG. Comparative effects of glibenclamide and metformin on ambulatory blood pressure and cardiovascular reactivity. *Diabetes Care* 1997; **20**: 692–697.
18. Campbell IW, Menzies DG, Chalmers J, McBain AM, Brown JRF. One year comparative trial of metformin and glipizide in Type 2 diabetes. *Diabete Metab* 1994; **21**: 394–400.
19. Hermann LS, Scherstén B, Bitzén P-O, Kjellström T, Lidgärfe F, Melander A. Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes Care* 1994; **17**: 1100–1109.
20. Hermann LS, Karlsson J-E, Sjöstrand A. Prospective comparative study in NIDDM patients of metformin and glibenclamide with special reference to lipid profiles. *Eur J Pharmacol* 1991; **41**: 263–265.
21. Rains SGH, Wilson GA, Elkeles RS. The effect of glibenclamide and metformin on serum lipoproteins in Type 2 diabetes. *Diabet Med* 1988; **5**: 653–658.
22. Relimpio F, Pumar A, Losada F, Mangas MA, Acosta D, Astorga R. Adding metformin versus insulin dose increase in insulin-treated but poorly controlled Type 2 diabetes mellitus: an open-label randomized trial. *Diabet Med* 1998; **15**: 997–1002.
23. Groop L, Widén E, Franssila-Kallunki A, *et al*. Different effects of insulin and oral antidiabetic agents on glucose and energy metabolism in Type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1989; **32**: 599–605.
24. Fanghanel G, Sanchez Reyes L, Trujillo C, Sotres D, Espinosa Campos J. Metformin's effect on glucose and lipid metabolism in patients with secondary failure to sulfonylureas. *Diabetes Care* 1996; **19**: 1185–1189.
25. Guigliano D, De Rosa N, Di Maro G, *et al*. Metformin improves glucose, lipid metabolism, and reduces blood pressure in

hypertensive, obese women. *Diabetes Care* 1993; **16**: 1387–1390.
26. Nagi DK, Yudkin JS. Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. A study of two ethnic groups. *Diabetes Care* 1993; **16**: 621–629.
27. Schneider J, Erren T, Zöfel P, Kaffarnik H. Metformin-induced changes in serum lipids, lipoproteins, and apoproteins in non-insulin-dependent diabetes mellitus. *Atherosclerosis* 1990; **82**: 97–103.
28. Giugliano D, Quatrano A, Consoli G, *et al*. Metformin for obese, insulin-treated diabetic patients: improvement in glycaemic control and reduction of metabolic risk factors. *Eur J Clin Pharmacol* 1993; **44**: 107–112.
29. Grant PJ. The effects of high- and medium-dose metformin therapy on cardiovascular risk factors in patients with Type II diabetes. *Diabetes Care* 1996; **19**: 64–66.
30. Jeppesen J, Zhou M-Y, Chen IY-D, Reaven G. Effect of metformin on postprandial lipemia in patients with fairly to poorly controlled NIDDM. *Diabetes Care* 1994; **17**: 1093–1099.
31. Grosskopf I, Ringel Y, Charach G, *et al*. Merformin enhances clearance of chylomicrons and chylomicron remnants in nondiabetic mildly overweight glucose-intolerant subjects. *Diabetes Care* 1997; **20**: 1598–1602.
32. Abbasi F, Kamath V, Rizvi AA, Carantoni M, Chen Y-DI, Reaven GM. Results of a placebo-controlled study of the metabolic effects of the addition of metformin to sulfonylurea-treated patients. *Diabetes Care* 1997; **20**: 1863–1869.
33. Patsch JR, Miesenböck G, Hopferweiser T, *et al*. Relation of triglyceride metabolism and coronary artery disease. *Arterioscler Thromb* 1992; **12**: 1336–1345.
34. Simpson LA, Dwyer T, Simons J, *et al*. Chylomicrons and chylomicron remnants in coronary artery disease; a case-control study. *Artherosclerosis* 1987; **65**: 181–189.
35. Snorgaard O, Køber L, Carlsen J. The effect of metformin on blood pressure and metabolism in nondiabetic hypertensive patients. *J Intern Med* 1997; **242**: 407–412.
36. Gudbjörnsdottir S, Friberg P, Elam M, Attvall S, Lönnroth P, Wallin G. The effect of metformin and insulin on sympathetic nerve activity, norepinephrine spillover and blood pressure in obese, insulin resistant, normoglycaemic, hypertensive men. *Blood Pressure* 1994; **3**: 394–403.
37. Bailey C, Turner RC. Metformin. *N Engl J Med* 1996; **334**: 574–579.
38. Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum γ-glutamyltransferase and risk of NIDDM. *Diabetes Care* 1998; **21**: 732–737.

