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Metformin-like effect of *Salvia officinalis* (common sage): is it useful in diabetes prevention?

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Common sage (*Salvia officinalis* L.) is among the plants that are claimed to be beneficial to diabetic patients, and previous studies have suggested that some of its extracts have hypoglycaemic effects in normal and diabetic animals. In the present study, we aimed to verify the antidiabetic effects of an infusion (tea) of common sage, which is the most common form of this plant consumed. Replacing water with sage tea for 14 d lowered the fasting plasma glucose level in normal mice but had no effect on glucose clearance in response to an intraperitoneal glucose tolerance test. This indicated effects on gluconeogenesis at the level of the liver. Primary cultures of hepatocytes from healthy, sage-tea-drinking rats showed, after stimulation, a high glucose uptake capacity and decreased gluconeogenesis in response to glucagon. Essential oil from sage further increased hepatocyte sensitivity to insulin and inhibited gluconeogenesis. Overall, these effects resemble those of the pharmaceutical drug metformin, a known inhibitor of gluconeogenesis used in the treatment and prevention of type 2 diabetes mellitus. In primary cultures of rat hepatocytes isolated from streptozotocin (STZ)-induced diabetic rats, none of these activities was observed. The present results seem to indicate that sage tea does not possess antidiabetic effects at this level. However, its effects on fasting glucose levels in normal animals and its metformin-like effects on rat hepatocytes suggest that sage may be useful as a food supplement in the prevention of type 2 diabetes mellitus by lowering the plasma glucose of individuals at risk.

***Salvia officinalis* L.: Diabetes: Metformin: Rat hepatocyte: Hypoglycaemic effects**

Diabetes mellitus is a disease characterised by increased plasma glucose levels that is the result of an insufficient production of (type 1 diabetes) and/or decreased tissue response to (type 2 diabetes) the pancreatic hormone insulin. Type 1 diabetes involves an autoimmune disease in which, destruction of the insulin-secreting β-cells of the pancreas by the individual's immune system. In type 2 diabetes, insulin resistance in the peripheral tissues strains insulin secretion, which leads to subsequent failure of the β-cells of the pancreas (Klover & Mooney, 2004). Type 2 diabetes accounts for the majority (85–90 %) of cases and is likely to become even more prevalent over the coming decades because of the increasing rates of childhood and adult obesity and the tendency for developing countries to embrace Western lifestyles (Williams & Pickup, 2004). Nowadays, diabetes mellitus is a major public health concern that has in many countries attained epidemic proportions.

Glucose is an essential nutrient for the human body, and mechanisms of glucose homeostasis aim to maintain the blood glucose level within a narrow range, about 5–7 mmol/l (Klover & Mooney, 2004; Williams & Pickup, 2004). In healthy individuals, blood glucose concentrations are maintained by the balance between glucose entry into the circulation from intestinal absorption and glucose uptake into peripheral tissues such as muscle and adipose tissue. Circulating levels of insulin increase after meals, stimulating GLUT-4-mediated glucose uptake by the peripheral tissues, thereby preventing hyperglycaemia. During periods when there is no intestinal glucose absorption, blood levels do not decrease drastically because the liver releases glucose into the circulation in response to the counter-regulatory pancreatic hormone glucagon, which stimulates both glycogen breakdown (glycogenolysis) and gluconeogenesis (i.e. the formation of new glucose from substrates, for example glycerol, lactate and amino acids such as alanine).

The anti-hyperglycaemic effects of insulin include the suppression of glucose output from the liver, inhibiting both glycogenolysis and gluconeogenesis. In healthy individuals, relatively low concentrations of insulin are needed to suppress hepatic glucose output (Roden & Bernroider, 2003; Williams & Pickup, 2004). In type 2 diabetes, however, not only does hyperglycaemia exist postprandially, where it reveals the inability of insulin to increase peripheral glucose uptake, but also elevated blood glucose levels persist even during fasting owing to increased gluconeogenesis in the liver (Roden & Bernroider, 2003; Klover & Mooney, 2004).

Before the disease has become established, those individuals more at risk of developing type 2 diabetes show the first signs of abnormal glucose metabolism, such as impaired glucose tolerance and/or impaired fasting glucose (Simpson *et al.* 2003). This provides an asymptomatic period at the beginning of the progression of type 2 diabetes, during which preventive interventions can be applied. Previous studies have shown that

Abbreviations: EO, essential oil; FBS, fetal bovine serum; IpGTT, intraperitoneal glucose tolerance test; STZ, streptozotocin.

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changes in dietary habits and sedentary behaviour can reduce the progression from impaired glucose tolerance to type 2 diabetes by 50–60 % (Chiasson *et al.* 2002; Simpson *et al.* 2003). However, the difficulty of maintaining lifestyle changes in the long term justifies the need for pharmacotherapeutic support, and recent studies have shown beneficial effects of metformin and acarbose during the progression from impaired glucose tolerance to type 2 diabetes (Simpson *et al.* 2003). Metformin mainly inhibits gluconeogenesis, whereas acarbose reduces intestinal glucose absorption. But any pharmacological intervention in an asymptomatic population raises ethical considerations in addition to practical and economic issues.

Dietary supplements with glucose-lowering properties could provide a culturally acceptable and economically viable alternative to pharmaceutical interventions at this stage. However, in spite of growing interest in the effects of herbs and food supplements on glucose control in diabetes, information remains insufficient (Day, 1998; Yeh *et al.* 2003). Plants have for centuries been used in folk medicine, and their beneficial effects have been well described. *Salvia officinalis* L. (common sage) is among those which are reputed to possess antidiabetic properties (Baricevic & Bartol, 2000).

Recently, Alarcon-Aguilar *et al.* (2002) showed that a water ethanolic extract from *S. officinalis* injected intraperitoneally had hypoglycaemic effects in fasted normoglycaemic mice and in fasted mildly alloxan-induced diabetic mice. In addition, Eidi *et al.* (2005) showed that a sage methanolic extract given intraperitoneally significantly reduced serum glucose level in fasted streptozotocin (STZ)-induced diabetic rats without changes in insulin level. Sage has a high essential oil (EO) content (Giannouli & Kintzios, 2000). The EO has also been tested and proved to be hypoglycaemically active in normal and in alloxan-induced diabetic rats (Baricevic & Bartol, 2000) but not in STZ-induced diabetic rats (Eidi *et al.* 2005).

With the present study, we aimed to evaluate the hypoglycaemic properties of a sage infusion (hereafter referred to as sage tea), the most common form of human sage consumption, and to shed some light on possible mechanisms of action. In mice and rats treated for 14 d with sage tea, we evaluated *in vivo* the response to an intraperitoneal glucose tolerance test (ipGTT), and, in primary cultures of hepatocytes isolated from normal and STZ-induced diabetic rats, the effect on responses to glucose, insulin and glucagon. The *in vitro* effects of sage EO were also investigated.

Materials and methods

Chemicals

Collagenase (grade IV), William's medium E, Dulbecco's modified Eagle's medium, dexamethasone, insulin, glucagon, 1,1-dimethylbiguanide hydrochloride (metformin), STZ and Bradford reagent were purchased from Sigma-Aldrich (St Louis, MO, USA). Glucofix for glucose measurements was acquired from A. Menarini Diagnostics (Firenze, Italy). All others reagents were of analytical grade.

Plant material, preparation of sage tea, isolation of essential oil and analysis of its constituents

Salvia officinalis L. plants were grown in an experimental farm located in Arouca, Portugal, and were collected in April, 2001. The aerial parts of plants were lyophilised and kept at − 20°C. The sage tea was routinely prepared as in a previous study by pouring 150 ml boiling water onto 2 g dried plant material and allowing it to steep for 5 min (Lima *et al.* 2005). This preparation produced a 3·5 (SEM 0·1) mg extract dry weight per ml infusion, with rosmarinic acid (362 μg/ml infusion) and luteolin 7-glucoside (115·3 μg/ml infusion) as major phenolic compounds, and 1,8-cineole, *cis*-thujone, *trans*-thujone, camphor and borneol as the major volatile components (4·8 μg/ml infusion; Lima *et al.* 2005). The EO was obtained by hydrodistillation, and the compounds were identified by GC and GC–MS in a previous work (Lima *et al.* 2004). The EO included approximately sixty compounds, the most abundant being *cis*-thujone (17·4 %), *alpha*-humulene (13·3 %), 1,8-cineole (12·7 %), *E*-caryophyllene (8·5 %) and borneol (8·3 %; Lima *et al.* 2004).

Animals

Female Balb/c mice (8–10 weeks) and male Wistar rats (150–200 g) were purchased from Charles River Laboratories (Barcelona, Spain) and acclimated to our laboratory animal facilities for at least 1 week before the start of the experiments. During this period, the animals were maintained on a natural light–dark cycle at 20 (SD 2) °C and given food and tap water *ad libitum*. The animals used in the experiments were kept and handled in accordance with our university regulations, which follow the *Principles of Laboratory Animal Care* (National Institutes of Health, 1985).

To study the effects of sage-tea-drinking, sage tea was given to mice and rats *ad libitum* for 14 d as a replacement for their water, as previously performed (Lima *et al.* 2005). The volumes consumed were not significantly different between water and sage tea in both normal mice and rats (Lima *et al.* 2005). Diabetes was induced in rats by the intraperitoneal injection of a freshly prepared STZ solution (50 mg/kg in 0·1 M-citrate buffer, pH 4·5). Experiments with diabetic rats were carried out 1 week after STZ injection. During this period, diabetes was well established, with polydipsia, polyuria and non-fasting blood glucose levels of over 250 mg/dl. The animals were used in four different experiments.

Experiment 1

This experiment aimed to evaluate the hypoglycaemic potential of the sage tea in normal mice, an ipGTT being performed on animals from two different groups (water and sage-tea-drinking). Twenty female Balb/c mice were randomly divided into two groups, and given food and either tap water or sage tea *ad libitum* for 14 d (the beverage being renewed daily). On day 15, an ipGTT (intraperitoneal injection of 300 g glucose/l in physiological saline in a dose of 5·83 ml/kg mouse) was performed in mice that had been fasted for 3 h (half of the animals from each group being a control group, with an intraperitoneal injection of saline alone). Blood samples were collected 45 min after the intraperitoneal injection, and plasma was used for glucose measurements.

Experiment 2

In this experiment, primary cultures of rat hepatocytes from overnight fasted normal animals, in a medium with low

concentrations of glucose and a gluconeogenic substrate (lactate), were used to evaluate the modulation by sage tea of hepatocyte glucose production. Eight male Wistar rats were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 d (the beverage being renewed daily). Hepatocyte isolation was performed between 10.00 and 11.00 hours by collagenase perfusion, as previously described by Moldeus *et al.* (1978) with some modifications (Lima *et al.* 2004), from overnight-fasted normal animals. Cell viability was over 85 %, as estimated by a trypan blue exclusion test.

Cells were suspended in Dulbecco's modified Eagle's medium (containing 5·6 mM-glucose) supplemented with 10 mM-lactate, fetal bovine serum (FBS; 100 ml/l), 10^{-9} M-insulin and 10^{-9} M-dexamethasone, and were seeded onto six-well culture plates at a density of 1×10^6 cells/well. Cells were incubated at 37°C in a humidified incubator gassed with 50 ml/l CO_2/air. After plating (to allow for cell attachment), culture medium was replaced with Dulbecco's modified Eagle's medium supplemented with 10 mM-lactate, 100 ml FBS/l and none, one or more of the following compounds: 10^{-7} M-glucagon, 10^{-3} M-metformin and/or sage EO (4 nl/ml). After 24 h incubation, the medium was recovered for glucose quantification. Metformin was used as positive control.

Experiment 3

In this experiment, primary cultures of normal rat hepatocytes in media with high (11 and 22 mM) concentrations of glucose (to mimic postprandial and diabetic conditions) were used to evaluate the effect of the sage tea on the glucose consumption capacity of the cells. Eight male Wistar rats were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 d (the beverage being renewed daily).

Hepatocytes were isolated from normal animals as described above, and cells were suspended in William's medium E (containing 11 mM-glucose) supplemented with 100 ml FBS/l, 10^{-9} M-insulin and 10^{-9} M-dexamethasone, and seeded onto six-well culture plates at a density of 1×10^6 cells/well. Cells were incubated at 37°C in a humidified incubator gassed with 50 ml/l CO_2/air. After plating, culture medium was replaced with William's medium E supplemented with 100 ml FBS/l and none, one or more of the following compounds: glucose (to a final concentration of 22 mM), 10^{-7} M-insulin and/or 4 nl sage EO/ml. After 24 h incubation, the media were recovered for glucose quantification.

Experiment 4

In this experiment, primary cultures of hepatocytes from STZ-induced diabetic rats were used in media with low and high concentrations of glucose (both containing the gluconeogenic substrate lactate) to evaluate effects of sage-tea-drinking on cell glucose production. Eight STZ-induced diabetic rats (male Wistar) were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 d (the beverage being renewed daily). Because the diabetic rats were polydipsic, sage-tea-drinking animals were pair-fed with the non-diabetic animals given diluted sage tea, in order to ensure a similar intake of tea dry weight to that of the rats in experiments 2 and 3.

Hepatocyte isolation from diabetic animals were performed as above, and cells were suspended in Dulbecco's modified Eagle's medium containing either 5·6 mM or 22 mM-glucose, supplemented with 10 mM-lactate, 100 ml FBS/l, 10^{-9} M-insulin and 10^{-9} M-dexamethasone, and seeded onto six-well culture plates at a density of 1×10^6 cells/well. The culture plates were incubated at 37°C in a humidified incubator gassed with 50 ml/l CO_2/air. After plating, culture medium was replaced with Dulbecco's modified Eagle's medium supplemented with 10 mM-lactate, 100 ml FBS/l and none, one or more of the following compounds: glucose (to a final concentration of 22 mM), 10^{-7} M-insulin, 10^{-7} M-glucagon, 10^{-3} M-metformin and/or 4 nl sage EO/ml. After 24 h incubation, the medium was recovered for glucose quantification. Metformin was used as a positive control.

Plating periods of 24 h were used in cell cultures from normally fed animals for cell attachment. In an attempt to preserve the altered physiological conditions, introduced by both the fasting and the STZ-induced diabetes, cells were plated for 3 h before exposure to the different test conditions. In the fasted condition, the results of plating for 24 h are also presented for comparison.

In all experiments with rat hepatocytes, lactate dehydrogenase activity was measured in the media to ensure no toxicity of the treatment on the cell layer.

Biochemical analysis

The concentrations of glucose in the mouse plasma and culture media were measured using a colorimetric enzymatic method (Glucofix) following the manufacturer's specifications.

The lactate dehydrogenase activity of the culture media was used as an indicator of hepatocyte plasma membrane integrity. The activity of the enzyme was measured at 30°C by quantifying NADH consumption by continuous spectrophotometry on a plate reader (Spectra Max 340pc; Molecular Devices, Sunnyvale, CA, USA; Lima *et al.* 2005).

Protein content was measured with the Bradford reagent purchased from Sigma-Aldrich using bovine serum albumin as a standard.

Statistical analysis

Data are expressed as means with standard errors of the means (SEM). Two-way ANOVA followed by the Student–Newman–Keuls *post hoc* test (SigmaStat, version 2.03; SPSS Inc., San Rafael, CA, USA) was employed in experiment 1 to compare the effects of the *in vivo* beverage (water *v.* sage tea) and the ipGTT (intraperitoneal saline *v.* intraperitoneal glucose). In experiments 2, 3 and 4 (in which two replicates were used for each experimental condition), the same statistical test was employed to compare the effects of the *in vivo* beverage (water *v.* sage tea) and the *in vitro* treatments (in this case, when a significant effect was obtained, a paired student's *t* test was employed to find the differences between each treatment). *P* values $\leq 0{\cdot}05$ were considered statistically significant.

Results

Experiment 1

A period of 14 d of sage-tea-drinking significantly lowered fasting (3 h and 45 min) plasma glucose concentration from 8·8 mM to 6·8 mM ($P \leq 0{\cdot}01$) in normal mice (Table 1). In response to an ipGTT, a significant increase in plasma glucose was observed at 45 min in both groups (Table 1), although no differences were observed between the water and sage-tea-drinking groups.

Experiment 2

When hepatocytes from overnight fasted rats were plated with 5·6 mM-glucose- and 10 mM-lactate-containing medium, there was a release of glucose (mainly due to gluconeogenesis) into the medium. Hepatocyte glucose production increased in response to glucagon (Fig. 1(A)) in cells from water-drinking animals but was not statistically significant in cells from sage-tea-drinking animals (Fig. 1(A,B)). In general, hepatocyte glucose production (Fig. 1(A)) was lower in cells isolated from sage-tea-drinking rats than in those of water-drinking controls ($P \leq 0{\cdot}05$), and the difference became significant in the glucagon + EO groups (Fig. 1(A)). When incubated with sage EO, a significant decrease in hepatocyte glucose production was observed in both drinking groups (Fig. 1(A)). In co-incubations with glucagon, sage EO significantly decreased glucose production in response to glucagon (Fig. 1(A)).

Metformin (a known inhibitor of gluconeogenesis) significantly lowered hepatocyte glucose production capacity, even when co-incubated with glucagon, in cells from both water- and tea-drinking animals (Fig. 1(B)).

Experiment 3

Hepatocyte glucose consumption measured after 24 h incubation was higher in 22 mM-glucose medium (Fig. 2(B)) than in 11 mM-glucose medium (Fig. 2(A)), and increased in response to insulin. Glucose consumption was significantly higher ($P \leq 0{\cdot}01$) in cells isolated from tea dinking rats under all circumstances tested.

When the cells were incubated with sage EO, no significant differences in hepatocyte glucose consumption were obtained, although consumption was higher in cells isolated from the sage-tea-drinking animals. In co-incubations with insulin, sage EO significantly potentiated the hormone's effects on glucose consumption (Fig. 2(A,B)).

Experiment 4

In contrast with the situation for cells from healthy animals, when hepatocytes from STZ-induced diabetic rats were plated with medium containing 22 mM-glucose (and 10 mM-lactate), glucose production (and not consumption) was observed. There was no stimulation of glucose consumption by insulin (Fig. 3). Sage-tea-drinking did not modify this situation. In addition, EO did not inhibit hepatocyte glucose production. Only metformin was able to reduce the glucose production of hepatocytes isolated from diabetic water and sage-tea-drinking rats (Fig. 3).

When hepatocytes from STZ-induced diabetic rats were plated with 5·6 mM-glucose (and 10 mM-lactate) containing medium, glucose production was similar in cells isolated from both water and sage-tea-drinking rats (Fig. 4). Glucagon did not further stimulate glucose production (Fig. 4). As above, no effect was observed for EO. Once again, metformin significantly reduced (by about 60 %) hepatocyte glucose production in cells from both water and sage-tea-drinking rats (Fig. 4).

All the treatments in the primary cultures did not induce lactate dehydrogenase release to the medium, an indicator that there was no cell toxicity in any of the *in vitro* treatments.

Table 1. Plasma glucose concentration in mice in response to an intraperitoneal glucose tolerance test (ipGTT; 45 min) performed on mice fasted for 3 h previously treated with or without sage tea for 14 d

(Values are means and standard errors of the mean, *n* 5)

		Plasma glucose (mM)	
Group	*In vivo* beverage	Mean	SEM
Control	Water	8·8	0·6
	Sage tea	6·8**	0·4
IpGTT	Water	10·5 *	0·3
	Sage tea	10·4†††	0·4

Mean values were significantly different compared with the water + control group *$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$.

Mean values were significantly different when compared with the water + control group: ††† $P \leq 0{\cdot}001$.

Discussion

The present work shows that sage-tea-drinking significantly reduced fasting plasma glucose level in mice. This suggested an inhibition of gluconeogenesis and/or glycogenolysis in the liver. In agreement with this, rat overall hepatocyte glucose production was lower in cells isolated from sage-tea-drinking animals than controls. Furthermore, stimulation with glucagon did not increase gluconeogenesis significantly in cells from sage-tea-drinking animals. Sage EO, although not as effective as metformin, produced a significant decrease in hepatocyte gluconeogenesis. In addition, the response of the hepatocytes to insulin was significantly increased by sage EO. These data suggests a metformin-like effect for sage tea and in particular for the EO fraction of *Salvia officinalis* L. These effects were, however, not observed in hepatocytes isolated from STZ diabetic animals, in which only metformin, a drug used in the treatment and prevention of type 2 diabetes, was effective in reducing glucose production. The effects of metformin were not modified by sage-tea-drinking, which seems to imply that sage tea, although not effective in diabetics, would not interfere negatively with metformin therapy.

Although using a different extract and experimental methodology, the hypoglycaemic effects of sage have previously been reported by others (Alarcon-Aguilar *et al.* 2002; Eidi *et al.* 2005). Alarcon-Aguilar *et al.* (2002) showed that, 4 h after an intraperitoneal injection of a sage water ethanolic extract, blood glucose decreased significantly in fasted normal mice and in fasted mildly alloxan-diabetic mice, but not in fasted severely alloxan-diabetic mice. Although the authors stated that insulin might have mediated the hypoglycaemic effect of

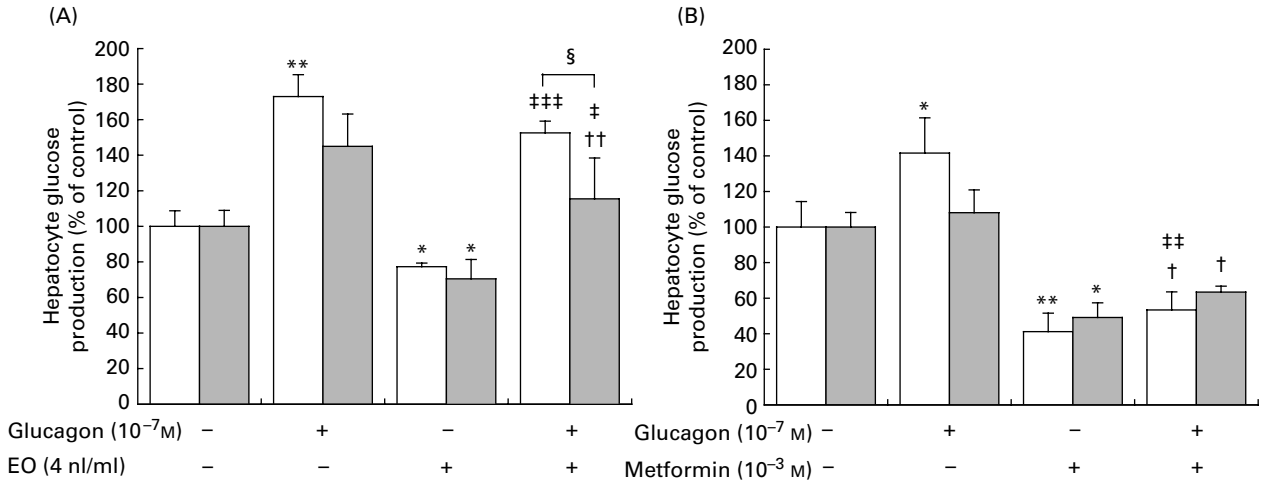

Fig. 1. Hepatocyte glucose production (24 h) by primary cultures of rat hepatocytes (isolated from overnight-fasted animals; □) and the effects of previous *in vivo* treatment with sage tea (for 14 d, ■) on hepatocyte responses to glucagon (10^{-7} M), essential oil (EO; 4 nl/ml) and metformin (10^{-3} M). The initial glucose concentration of the medium was 5·6 mM, and experiments were performed 24 h (A) or 3 h (B) after plating. Values were means with standard errors of the means shown by vertical bars (*n* 4). (A) Water-drinking rats: 100 % = 6·4 (SEM 0·6) μmol glucose/mg protein; sage-tea-drinking rats: 100 % = 6·2 (SEM 0·6) μmol glucose/mg protein. (B) Water-drinking rats: 100 % = 8·2 (SEM 1·2) μmol glucose/mg protein; sage-tea-drinking rats: 100 % = 9·5 (SEM 0·8) μmol glucose/mg protein. Mean values were significantly different compared with the respective control group: $^{*}P \leq 0{\cdot}05$, $^{**}P \leq 0{\cdot}01$. Mean values were significantly different compared with the respective glucagon group: $\dagger P \leq 0{\cdot}05$, $\dagger\dagger P \leq 0{\cdot}01$. Mean values were significantly different compared with the respective EO group (A) or metformin group (B): $\ddagger P \leq 0{\cdot}05$, $\ddagger\ddagger P \leq 0{\cdot}01$, $\ddagger\ddagger\ddagger P \leq 0{\cdot}001$. Mean value was significantly different between the water and sage tea groups: $\S P \leq 0{\cdot}05$.

the extract, once the animals were tested in the fasted condition, it seems likely that an inhibition of gluconeogenesis was the cause of the effects observed in their study, as indeed suggested by the present results. Additionally, Eidi *et al.* (2005) showed that, 3 h after an intraperitoneal injection of a sage methanolic extract, blood glucose decreased significantly in fasted STZ-diabetic rats but not in fasted normal rats. This effect was not accompanied by an increased release of insulin (Eidi *et al.* 2005).

In human subjects, the abnormal glucose metabolism observed in both prediabetic states and in overt type 2 diabetes results in part from a deregulation of glucose production by the liver, which is mainly caused by an unrestrained glucagon stimulation of gluconeogenesis. In these individuals, therefore,

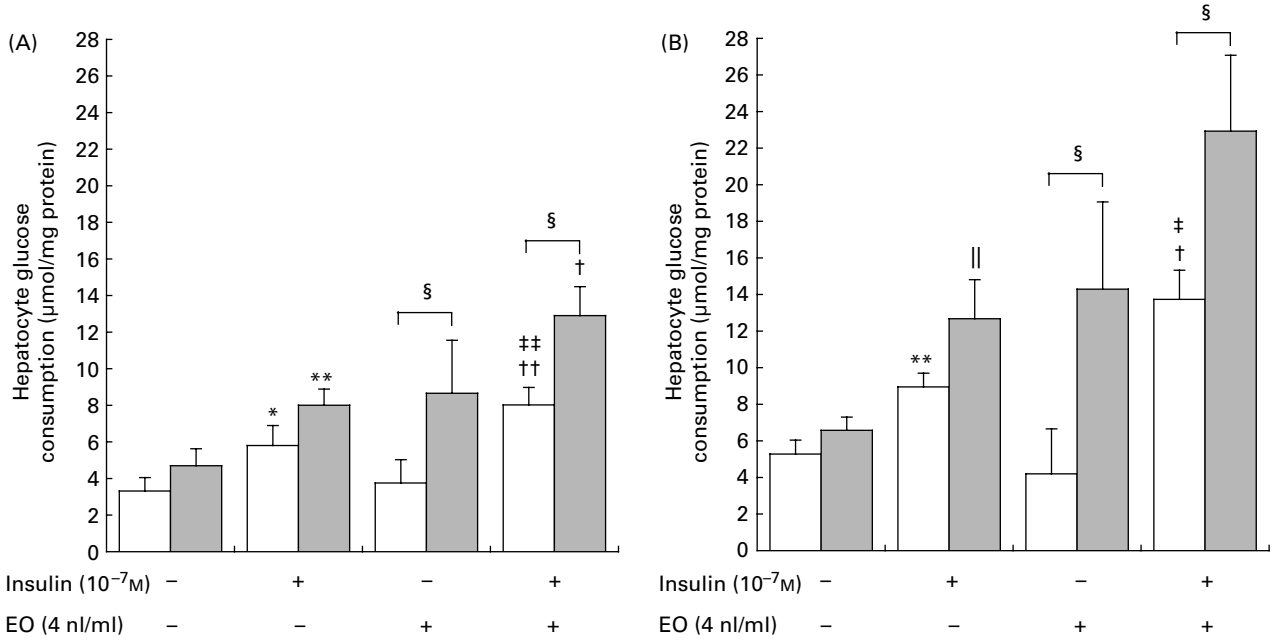

Fig. 2. Glucose consumption (24 h) by rat hepatocytes in primary cultures (□), and effects of previous *in vivo* treatment with sage tea (for 14 d; ■) on hepatocyte responses to insulin (10^{-7} M) and essential oil (EO; 4 nl/ml). The initial glucose concentrations of the medium were 11 mM (A) and 22 mM (B). Values were means with standard errors of the means shown by vertical bars, *n* 4. Mean values were significantly different compared with the respective control group: $^{*}P \leq 0{\cdot}05$, $^{**}P \leq 0{\cdot}01$. Mean values were significantly different compared with the respective insulin group: $\dagger P < 0{\cdot}05$, $\dagger\dagger P \leq 0{\cdot}01$. Mean values were significantly different compared with the respective EO group $\ddagger P \leq 0{\cdot}05$, $\ddagger\ddagger P \leq 0{\cdot}01$. Mean value was significantly different between the water and sage tea gruops: $\S P \leq 0{\cdot}05$. Mean value was almost significant compared with respective control group: $\| P = 0.573$.

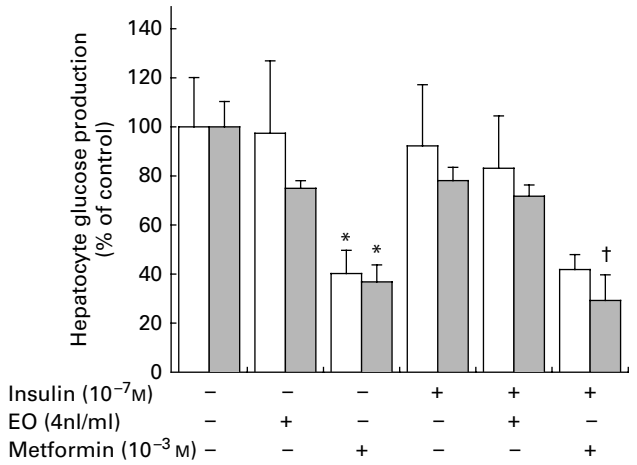

Fig. 3. Hepatocyte glucose production (24 h) by primary cultures of rat hepatocytes (isolated from streptozotocin-induced diabetic animals; □), and the effects of previous *in vivo* treatment with sage tea (for 14 d; ■) on hepatocyte responses to insulin (10^{-7} M), essential oil (EO; 4 nl/ml) and metformin (10^{-3} M). The initial glucose concentrations of the medium were 22 mM, and experiments were performed 3 h after plating. Values were means with standard errors of the means shown by vertical bars, *n* 4. Water-drinking rats: 100 % = 8·4 (SEM 1·7) μmol glucose/mg protein, sage-tea-drinking rats: 100 % = 8·7 (SEM 0·9) μmol glucose/mg protein. Mean values were significantly different compared with the respective control group: $^{*}P \leq 0{\cdot}05$. Mean values was significantly different compared with the respective insulin group: †$P \leq 0{\cdot}05$.

gluconeogenesis is active even when plasma glucose concentrations are already elevated, which further aggravates hyperglycaemia (Roden & Bernroider, 2003). By analogy with the effects of the drug metformin, used in the prevention and treatment of diabetes, the observed decrease in hepatocyte glucose production of sage-tea-drinking animals could be favourable, by

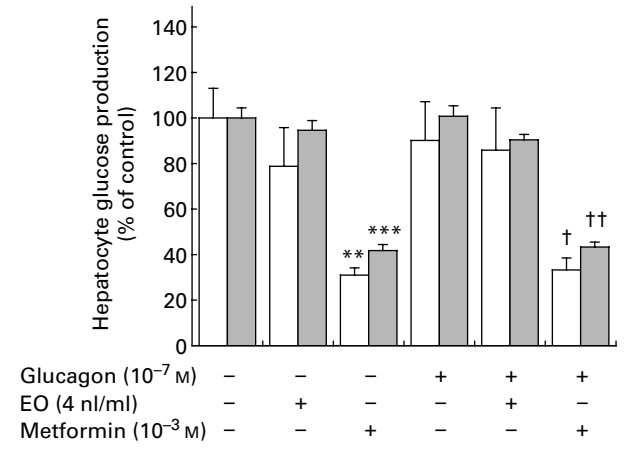

Fig. 4. Hepatocyte glucose production (24 h) by primary cultures of rat hepatocytes (isolated from streptozotocin-induced diabetic animals; □), and the effects of previous *in vivo* treatment with sage tea (for 14 d; ■) on hepatocyte responses to glucagon (10^{-7} M), essential oil (EO; 4 nl/ml) and metformin (10^{-3} M). The initial glucose concentration of the medium was 5·6 mM, and experiments were performed 3 h after plating. Values were means with standard errors of the means shown by vertical bars, *n* 4. Water-drinking rats: 100 % = 7·9 (SEM 1·0) μmol glucose/mg protein, sage-tea-drinking rats: 100 % = 7·6 (SEM 0·3) μmol glucose/mg protein). Mean values were significantly different compared with the respective control group: $^{**}P \leq 0{\cdot}01$, $^{***}P \leq 0{\cdot}001$. Mean values were significantly different compared with the respective glucagon group: †$P \leq 0{\cdot}05$, ††$P \leq 0{\cdot}001$.

preventing the liver's contribution to hyperglycaemia in groups at risk. Metformin is a derivative of guanidine, the active compound of goat's rue (*Galega officinalis*; Williams & Pickup, 2004). It acts by reducing liver glucose production and increasing the action of insulin (Chiasson *et al.* 2002). Also, in the present experiments, metformin showed these effects in both hepatocytes isolated from normal and STZ diabetic rats.

Generally, sage-tea-drinking increased rat hepatocyte glucose consumption, decreased fasting gluconeogenesis and inhibited the stimulation of hepatic glucose production by glucagon. However, in spite of decreasing plasma glucose, sage tea did not, after an *in vivo* ipGTT in mice, improve glucose clearance, which suggests that sage tea did not increase the insulin response *in vivo*. In addition, the stimulatory effects of *in vitro* insulin on glucose consumption were observed only in co-incubations with EO. This suggests a role for EO in the increase in sensitivity to insulin. Increased insulin sensitivity has been suggested as a possible mechanism of action of other plant extracts with attributed hypoglycaemic activities (Li *et al.* 2004; Qin *et al.* 2004; Saxena & Vikram, 2004; Han *et al.* 2005). The presence of low amounts of EO in sage tea, about 4·8 μg/ml (Lima *et al.* 2004), could explain the lack of an increase in insulin sensitivity observed after tea-drinking both *in vivo* and *in vitro*. We cannot exclude the fact that higher doses of the tested compounds administered by either gavage or intraperitoneal injection could produce similar effects as *in vitro*.

Also, in STZ-induced diabetic rat hepatocytes, sage-tea-drinking and EO *in vitro* lead to no significant improvement in the response to insulin. The liver (and hepatocytes) usually suppresses glucose release in response to insulin (Klover & Mooney, 2004). In STZ rat hepatocytes, insulin administration failed to suppress glucose production. Previous studies have also indicated that insulin is incapable of stimulating glucose utilisation *in vitro* by hepatocytes from STZ-induced diabetic rats (Salhanick *et al.* 1983; Amatruda *et al.* 1984; Hussin & Skett, 1988). The insulin resistance imposed by STZ treatment was not reversed by sage tea and/or EO. The lack of effect of sage tea/EO on STZ hepatocytes seems to indicate that sage requires an intact insulin signalling pathway to produce its effects. In the STZ diabetic rat, stimulation of the hepatocytes with glucagon did not enhance gluconeogenesis. Others have also failed to significantly stimulate gluconeogenesis *in vitro* in hepatocytes from STZ-induced diabetic rats (Dunbar *et al.* 1989).

One possibility for the lack of glucagon stimulation of gluconeogenesis in diabetic hepatocytes is that gluconeogenesis, *in vivo*, had been maximally stimulated. Cells are, however, metabolically competent and respond to metformin with a decrease in glucose production. In STZ-treated rats, insulin deficiency increases gluconeogenesis through enhanced lactate and pyruvate uptake and flux through the enzyme phosphoenolpyruvate carboxykinase (Large & Beylot, 1999). Metformin has been shown to reduce substrate flux through this enzyme (Large & Beylot, 1999) and to inhibit phosphoenolpyruvate carboxykinase gene expression (Cheng *et al.* 2001; Yuan *et al.* 2002), thereby decreasing gluconeogenesis. This inhibition of gene expression seems to occur mainly through an insulin-independent pathway (Yuan *et al.* 2002). This agrees with the possibility that sage tea and/or sage EO requires an intact insulin signalling pathway to produce its effects, which were observed only in normal rats.

The reduction in fasting plasma glucose shown in healthy animals indicates the potential for sage extracts to help prevent type 2 diabetes through a metformin-like effect, mainly in people at risk of developing it, as is the case of those who present impaired glucose tolerance and impaired fasting glucose. Taking into consideration the high worldwide, and increasing, prevalence of type 2 diabetes and the high costs involved in its treatment, the primary prevention of this disease is an important issue (Lai, 2002; Costacou & Mayer-Davis, 2003; Jermendy, 2005). Considering that there is now substantial evidence that type 2 diabetes could be considered to be a preventable disease through changes in lifestyle that include, among others, dietary factors (Costacou & Mayer-Davis, 2003; Schernthaner, 2003; Simpson *et al.* 2003; Stoeckli & Keller, 2004; Jermendy, 2005), the search for preventive strategies should be actively promoted. Sage products can easily be considered as functional foods or food supplements that could have a beneficial impact in low-cost prevention strategies for diabetes. In addition, there are health benefits from the use of plant extracts as sources of antioxidants. With particular regard to the liver, sage tea has been shown not to have toxic effects and to improve liver glutathione levels (Lima *et al.* 2005). Among other effects, this may indirectly improve the liver-mediated insulin response *in vivo* (Guarino *et al.* 2003). We are currently undertaking a pilot study with human volunteers to test the effects on the control of glycaemia in individuals at risk of developing diabetes. Experiments with animal models of type 2 diabetes, such as the Zucker rat (Sreenan *et al.* 1996), could also provide additional information on the therapeutic effects of sage tea.

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