BMC Clinical Pharmacology

BioMed Central

Research article

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Assessment of single-dose benzodiazepines on insulin secretion, insulin sensitivity and glucose effectiveness in healthy volunteers: a double-blind, placebo-controlled, randomized cross-over trial [ISRCTN08745124]

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Abstract

Background: The present study aimed at investigating in healthy volunteers the effects of diazepam and clonazepam on beta-cell function, insulin sensitivity and glucose effectiveness based on the frequently sampled intravenous (0.5 gkg^{-1}) glucose tolerance test with minimal-model analysis.

Methods: The study was designed as a double-blind, placebo-controlled, cross-over clinical trial. Diazepam (10 mg) and clonazepam (1 mg) were infused during 30 min to 15 male subjects with a mean age of 22 years (range: 20–29), after informed consent was given. Benzodiazepines were assayed by capillary gas chromatography with electron capture, insulin by radioimmunoassay and glucose by the enzymatic glucose oxidase method.

Results: Both benzodiazepines induced significant psychotropic effects. The acute insulin responses (AIR) were significantly and negatively correlated with the clonazepam plasma concentrations (r = -0.609, P < 0.05, n = 14). However, the mean AIR was not significantly different between the benzodiazepine-treated subjects and the controls. In addition, the parameters of glucose assimilation were significantly decreased as compared with placebo in the subgroup of 7 subjects with plasma clonazepam concentrations higher than 6.0 ng ml^{-1} (median and lower limit of effective therapeutic concentrations): 1.37 ± 0.3 *versus* 2.84 ± 0.60 × $10^{-2}min^{-1}$ (P = 0.028) for the coefficient of glucose tolerance (K_g), 2.18 ± 0.29 *versus* 3.71 ± 0.89 × $10^{-4}\mu Uml^{-1}min^{-1}$ (P = 0.018) for insulin sensitivity (S_i) and 1.80 ± 0.39 *versus* 3.59 ± 0.71 × $10^{-2}min^{-1}$ (P = 0.028) for glucose effectiveness at basal insulin (S_g). These parameters were not significantly modified when diazepam was administered; plasma levels of this drug however, were below the effective therapeutic concentrations (300 ng ml^{-1}) from min 15 after the end of the perfusion.

Conclusion: The present results suggest that a benzodiazepine, in particular clonazepam, may alter insulin secretion and insulin sensitivity after a single administration in healthy volunteers.

Background

Benzodiazepine drugs are widely prescribed, mainly for their anxiolytic and sedative properties. In addition to these psychotropic actions, different metabolic effects have also been reported but few clinical trials on that topic are available.

A trend to increased glycemia without significant modification of insulinemia was shown after a single dose administration of diazepam in healthy volunteers [1]. Aggravation of hyperglycemia has been reported during benzodiazepine treatment in diabetic patients [2]. In patients treated with the imidazopyridine compound alpidem, which binds benzodiazepine receptors [3], we reported an alteration of glucose tolerance after one week administration [4]. Studies *in vitro* have shown that some benzodiazepines could affect insulin secretion differently according to the experimental model [5,6].

Weight gain, which is often related to insulin resistance, has also been claimed to occur in some patients treated with benzodiazepines. Different benzodiazepines have different effects on body weight gain in rats, suggesting that different subtypes of benzodiazepine receptors are involved [7].

In order to further explore the potential effects of benzodiazepines on beta-cell function and insulin sensitivity, we performed in healthy volunteers the frequently sampled intravenous glucose tolerance test with minimal-model analysis according to Bergman *et al.* [8]. The single-dose effects of two benzodiazepines, diazepam and clonazepam, with different affinity and selectivity for central and peripheral receptors were investigated.

Methods

Fifteen healthy male volunteers, aged 20–29 years (mean 22.5 years), were included in this study. They underwent a full medical examination with electrocardiogram and standard biological screening before entry into the study. All subjects were in good physical condition and of normal weight (mean: 68.6 kg; range: 55–81) and body mass index (mean: 22.3 kg/m^2; range: 20.1–25.0). They had no previous history of significant disease and satisfied the inclusion/exclusion criteria. All volunteers gave written consent to participate after being informed of the aim of the research, its experimental protocol and potential risks. The study was approved by the institutional Ethics Committee of Montpellier and has been conducted in accordance with the Helsinki declaration and the European guideline for « Good Clinical Practice ».

The study was designed as a double-blind, three-way placebo-controlled cross-over investigation of diazepam, a central and peripheral benzodiazepine receptor ligand [9], clonazepam, a selective central receptor ligand [9], and placebo. Subjects were randomly assigned to one of the three treatment groups to control for potential order effect. The three treatments were single intravenous doses of diazepam (10 mg), clonazepam (1 mg) or placebo. The three treatment administrations were separated by a 7-day washout period.

In the morning of each test-day, subjects were admitted to the Clinical Investigation Center, in fasting conditions for at least 12 hours, namely from the evening before. The subjects remained in the supine position and a venous catheter was inserted in each forearm, one for the administration of the drugs and glucose, the other for blood sampling. The drugs in 125 mL saline solution or the saline solution alone (placebo) were administered during 30 min (between minutes -45 and -15). The treatments were prepared by the research nurse according to the randomization form. Fifteen minutes after the end of the perfusion, the frequently sampled intravenous glucose tolerance test (FSIVGTT) was started, with a glucose load of 0.5 g/kg in 3 min and a bolus of 0.03 U/kg insulin just before minute 20. Blood samples were taken at times -45, -15 and 0, then at 1, 3, 4, 6, 8, 10, 15, 19, 20, 22, 24, 30, 41, 70, 90 and 180 minutes after glucose injection for the measurement of plasma glucose and serum insulin concentrations. Additional samples were taken at times -45, -15, 0, then at 15, 30, 60, 120 and 180 minutes after glucose injection for the measurement of plasma diazepam, N-desmethyldiazepam, clonazepam and cortisol.

Furthermore, the subjects' mood and feelings were self-rated before (time -45 min) and after the infusion (time -15 min), and then at times 70 and 180 minutes, using the 49-item questionnaire from the Addiction Research Center Inventory (ARCI) and the Norris series of 16 bipolar visual analogue scales (VAS). At the end of the test day, subjects were discharged after a last clinical evaluation.

After immediate centrifugation of the blood samples (1500 g, 10 min), plasma or serum was separated and frozen at -20°C. Plasma glucose levels were assayed by the glucose oxidase method derived from Trinder [10]. Serum insulin and plasma cortisol were measured by radioimmunoassay using the marketed immunoradiometric kit INSIK-5 purchased from DiaSorin (Saluggia, Italy). Diazepam, N-desmethyldiazepam and clonazepam were assayed by a capillary gas chromatography technique with electron capture, adapted from a previously published method [11]. Briefly, benzodiazepines were extracted from plasma at pH = 9.0 by ethyl ether. GLC analysis was performed on a HP-5 (crosslinked 5 % PH ME siloxane) 30 m × 0.32 mm × 0.25 µm capillary column (Agilent) at an oven temperature from 170 to 250°C (2°C/min). The intra and inter-assay variability ranged

from 4.7 % to 9.9 % for clonazepam, 2.7 % to 7.4 % for diazepam and 5.4 % to 9.7 % for N-desmethyldiazepam for three quality control concentrations.

Acute insulin response (AIR) was used as an index of the first-phase insulin secretion and was calculated as the mean of the serum insulin concentrations at times 1, 3, 4, 6, 8 and 10 minutes.

The least-square slope of the log of the absolute glucose concentration between 4 and 19 minutes after the glucose bolus was used as an index of glucose tolerance (K_g).

Minimal-model analysis of the FSIVGTT was performed according to the method previously reported by Bergman *et al.* [8] and Yang *et al.* [12], using the software TISPAG from the Department of Clinical Physiology, Lapeyronie University Hospital, (Montpellier, France), which uses a nonlinear least-square estimation [13]. This program produced the values for insulin sensitivity (S_i) and glucose effectiveness (S_g). S_i and S_g are calculated from the equations $dG(t)/dt = -[p1 + X(t)]G(t) + p1Gb$, $G(0) = Go$, $dX(t)/dt = -p2X(t) + p3 [I(t) - Ib]$, and $X(0) = 0$, where $G(t)$ and $I(t)$ are plasma glucose and insulin concentrations, $X(t)$ is the insulin in a compartment remote from plasma (insulin action), and p1 to p3 are model parameters. Go is the glucose concentration that would be obtained immediately after injection if there was instantaneous mixing in the extracellular fluid compartment. Gb and Ib are basal values of glucose and insulin. Parameter p1 represents S_g, i.e. the fractional disappearance rate of glucose independent of any insulin response, and p3 and p2 determine the kinetics of insulin transport, respectively, into and out the remote insulin compartment where insulin action is expressed. S_i is an index of the influence of plasma insulin to change the glucose effect *per se* on glucose concentration. Thus, S_i is equal to -p3/p2.

S_g was divided into its two components: the contribution of hyperglycemia *per se* to tissue glucose utilization and the effect of basal insulin on glucose uptake. The basal insulin component of S_g is BIE and can be calculated as the product of basal insulin Ib and S_i: $BIE = Ib \times S_i$. Thus, the contribution of non-insulin-dependent glucose uptake (GEZI) to glucose uptake is the difference between total S_g and the BIE: $GEZI = S_g - (Ib \times S_i)$.

The sample size was calculated from clinical data on AIR obtained in a control sample after FSIVGTT, with the 2-sided hypothesis of a difference between diazepam and placebo or clonazepam and placebo of at least 35%; the study power was set at 90% and the significance threshold at 5%. All the results are given as means ± standard error (s.e. mean). Where necessary, 95% confidence intervals on differences (95% CI) are given. The time courses of

mean cortisol concentrations were compared using repeated measures ANOVA. Other kinetics data were integrated in areas under the curves (AUC), calculated including baseline by the trapezoidal rule using the Systat® 6.0 software for Windows®. AUC means were compared using the nonparametric Friedman test for related data. Spearman correlations were performed between AIR and diazepam or clonazepam concentrations. *Post hoc* and subgroups analysis were performed where necessary using the paired *t*-test for cortisol and the Wilcoxon rank-sum test for other paired data. The Systat® 6.0 software for Windows® was also used for the statistical analysis.

Results
Intravenous administration of 10 mg diazepam during 30 min yielded to plasma concentrations peaking at 370.1 ± 22.6 ngml^{-1} at time -15 min, just at the end of the infusion. The concentrations rapidly decreased to 229.7 ± 12.6 ngml^{-1} at time 0, before glucose administration (Figure 1, A). The mean concentrations of N-desmethyldiazepam ranged between 3.2 ± 0.9 ngml^{-1} at min -15 and 9.1 ± 1.6 ngml^{-1} at min 180. Intravenous infusion of 1 mg clonazepam over 30 min led to a mean maximal concentration of 15.1 ± 2.0 ngml^{-1} at min -15 (Figure 1, B); the mean concentrations at times 0 and 15 minutes were 6.6 ± 0.5 and 4.9 ± 0.4 ngml^{-1}, respectively.

Concerning the psychotropic effects of the drugs, the pentobarbital-chlorpromazine-alcohol group (PCAG) scores of the ARCI, which reflect sedation, were significantly different with treatment (P < 0.001, Figure 2); a significant increase was observed for diazepam (+363% *vs* placebo, 95% CI: 214.0, 820.7, n = 15, P = 0.004) and clonazepam (+593% *vs* placebo, 95% CI: 511.2, 1146.3, n = 14, P = 0.001). The benzedrine group (BG) scores, which explore stimulation, were also significantly different with treatment (P < 0.017, Figure 2); a significant decrease was observed for clonazepam (-153% *vs* placebo, 95% CI: -598.2, -170.3, n = 14, P = 0.004). Concerning the other factors of the ARCI, the scores of the morphine-benzedrine group (MBG), assessing euphoria, of the LSD group, assessing dysphoric and psychotomimetic changes, and of the amphetamine group, assessing amphetamine-like effects were not significantly modified with treatment (not shown). On the VAS, the self-rating of asthenia-fatigue (factor 1) scores were also significantly modified with treatment (P = 0.002), with a similar time-course as the PCAG score of the ARCI (not shown); a significant increase was observed for diazepam (+42.3% *vs* placebo, 95% CI: 899.1, 4501.6, n = 15, P = 0.015) and clonazepam (+70.3% *vs* placebo, 95% CI: 2402.2, 6516.4, n = 14, P = 0.002).

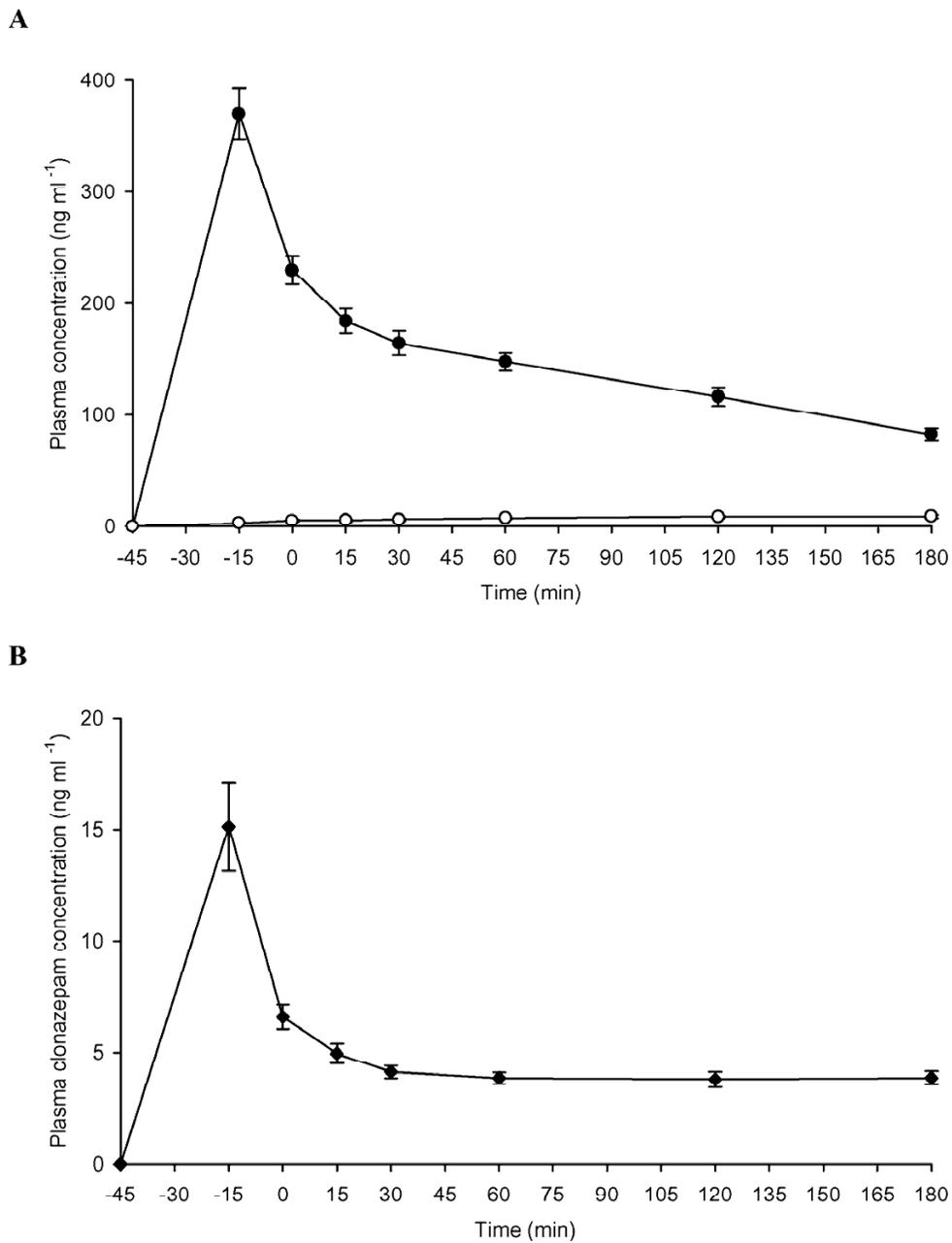In addition, there was a significant difference (P = 0.028) in the time course of plasma cortisol concentrations

Figure 1
Plasma concentrations after diazepam and clonazepam infusion. A Time course of plasma diazepam and N-desmethyldiazepam concentrations after 10 mg diazepam infusion (during 30 min from time -45 min to time -15 min) duri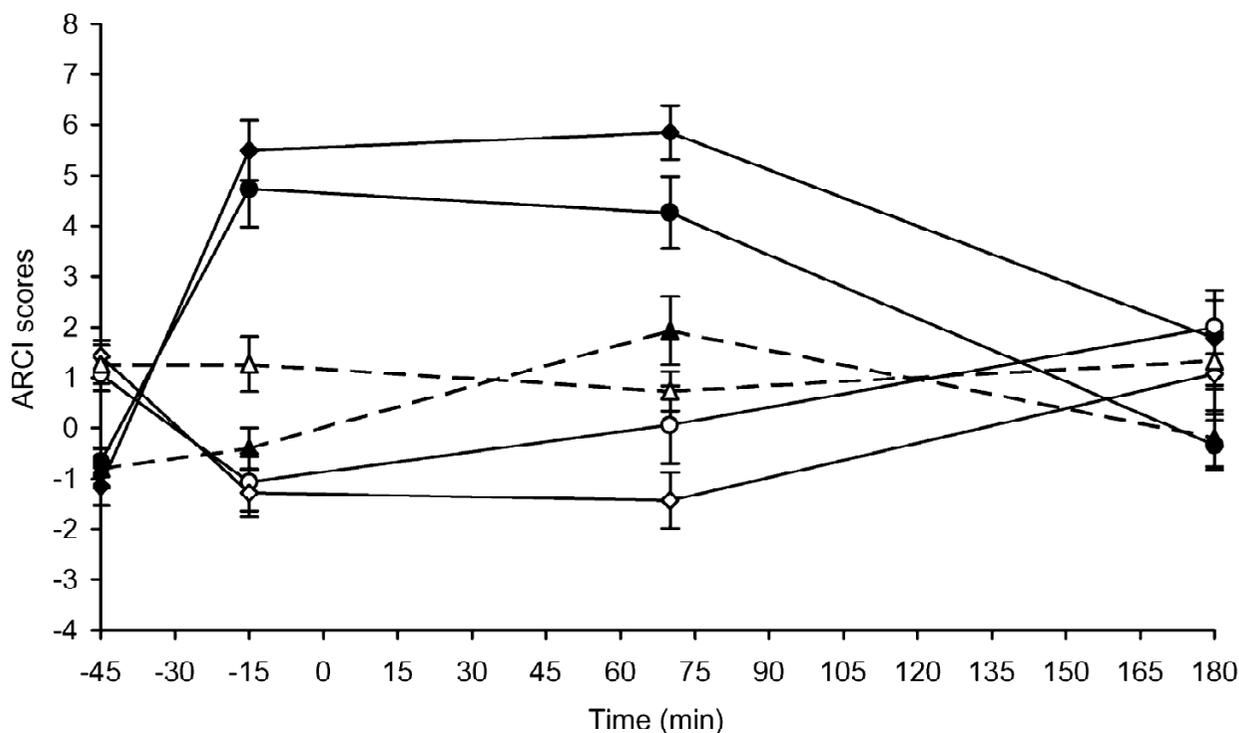ng FSIVGTT (0.5 g/kg glucose load at time 0 min). ● diazepam; ○ N-desmethyldiazepam. B Time course of plasma clonazepam concentration after 1 mg clonazepam infusion (during 30 min from time -45 min to time -15 min) during FSIVGTT (0.5 g/kg glucose load at time 0 min). ◆ clonazepam.

Figure 2
Time course of PCAG- and BG-scores of the ARCI after 10 mg diazepam, 1 mg clonazepam or placebo infusion (during 30 min from time -45 min to time -15 min) during FSIVGTT (0.5 g/kg glucose load at time 0 min). ● PCAG scores after diazepam infusion; ◆ PCAG scores after clonazepam infusion; ▲ PCAG scores after placebo infusion; ○ BG scores after diazepam infusion; ◇ BG scores after clonazepam infusion; △ BG scores after placebo infusion.

between the different groups (Figure 3); a significant reduction was observed at time 60 min for diazepam (-33.3% *vs* placebo, 95% CI: -130.4, -38.3, n = 15, P = 0.002) and clonazepam (-24.0% *vs* placebo, 95% CI: -119.0, -2.6, n = 15, P = 0.042).

As shown in figure 4, the acute insulin responses (AIR) were significantly and negatively correlated with the plasma concentrations of clonazepam (r = -0.609, P < 0.05, n = 14) at time 0 minute, just before glucose injection. However, the mean AIR were not significantly different between the benzodiazepine-treated subjects and the controls (Table 1).

Overall, glucose tolerance, insulin sensitivity and glucose effectiveness were not significantly different between the benzodiazepine-treated subjects and the controls (Table 1). However, as shown in figure 5, a concentration-dependent effect could be observed with clonazepam. Indeed, in the subgroup of 7 subjects with plasma clonazepam concentrations at time 0 minute higher than 6.0 ngml^{-1} (median and lower limit of effective therapeutic concentrations), each of these parameters was significantly decreased as compared with placebo: -51.8% (95% CI: -2.58, -0.37 × 10^{-2}min^{-1}, P = 0.028) for the coefficient of glucose tolerance (K$_g$), -41.2% (95% CI: -3.04, -0.02 × 10^{-4}µUml^{-1}min^{-1}, P = 0.018) for insulin sensitivity (S$_i$) and -49.9% (95% CI: -3.07, -0.51 × 10^{-2}min^{-1}, P = 0.028) for glucose effectiveness at basal insulin (S$_g$).
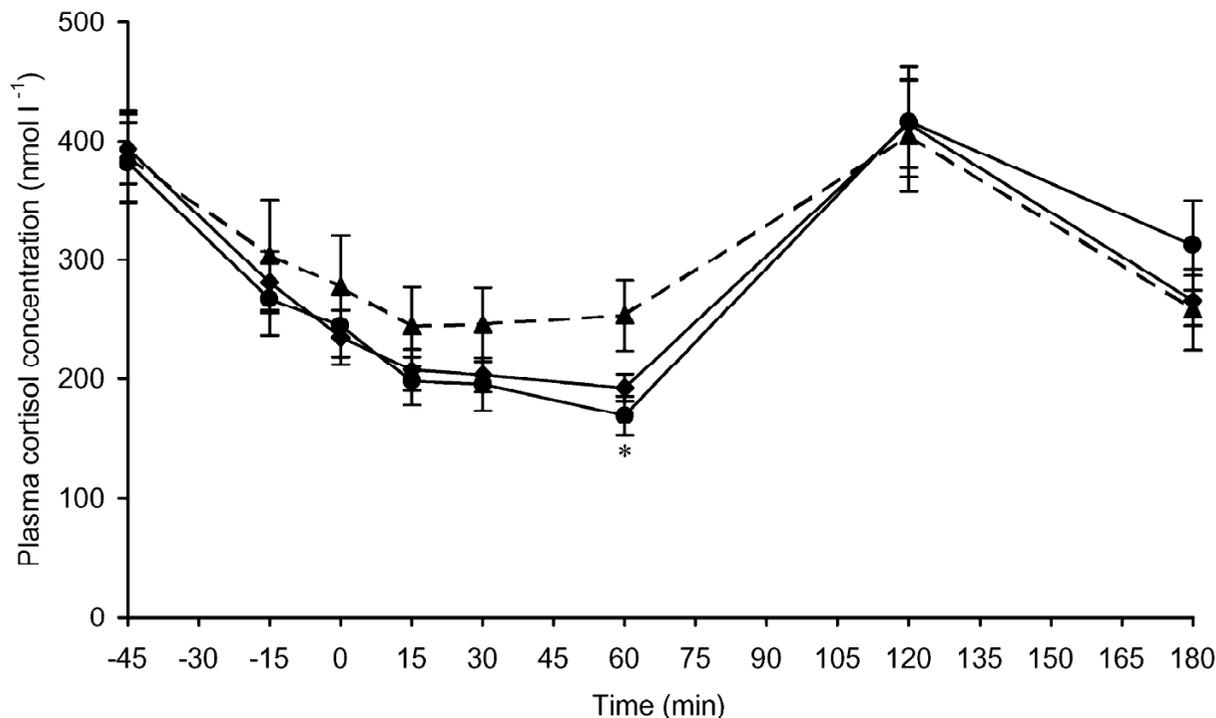
Figure 3
Time course of plasma cortisol after 10 mg diazepam, 1 mg clonazepam or placebo infusion (during 30 min from time -45 min to time -15 min) during FSIVGTT (0.5 g/kg glucose load at time 0 min). ● diazepam; ◆ clonazepam; ▲ placebo; * P < 0.05 for diazepam *vs* placebo and clonazepam *vs* placebo.

Furthermore, the two components of S_g were significantly decreased with clonazepam *vs* placebo in the subgroup of subjects with the highest plasma clonazepam concentrations at time 0 minute: -38.1% (95% CI: -0.18, -0.03 × 10^{-2}min^{-1}, P = 0.018) for basal insulin effctiveness (BIE) and -50.9% (95% CI: -2.97, -0.40 × 10^{-2}min^{-1}, P = 0.028) for glucose effectiveness at zero insulin (GEZI).

Discussion
The present study investigated the effects of a single-dose administration of different benzodiazepines on β-cell function and insulin sensitivity in healthy volunteers.

Intravenous administration of 10 mg diazepam or 1 mg clonazepam led to peak concentrations at the end of the infusion within the standard effective plasma concentra-tions of these drugs, namely 300–400 ngml^{-1} for diazepam and 5–70 ngml^{-1} for clonazepam [14]. How-ever, whereas the plasma levels of diazepam rapidly decreased below the standard effective concentrations, the mean concentrations of clonazepam further remained around the lower limit of effective concentrations.

Both diazepam and clonazepam induced significant psy-chological effects, as judged by the PCAG and BG scores of the ARCI and the asthenia-fatigue factor of the VAS, which is in accordance with previously reported data on the subjective effects induced by benzodiazepines [15]. These effects were still significant at time 70 minutes, ie approx-imately one and a half hour after the end of the infusion, although at that time, the PCAG and VAS factor 1 scores were also increased in the placebo group. This latter
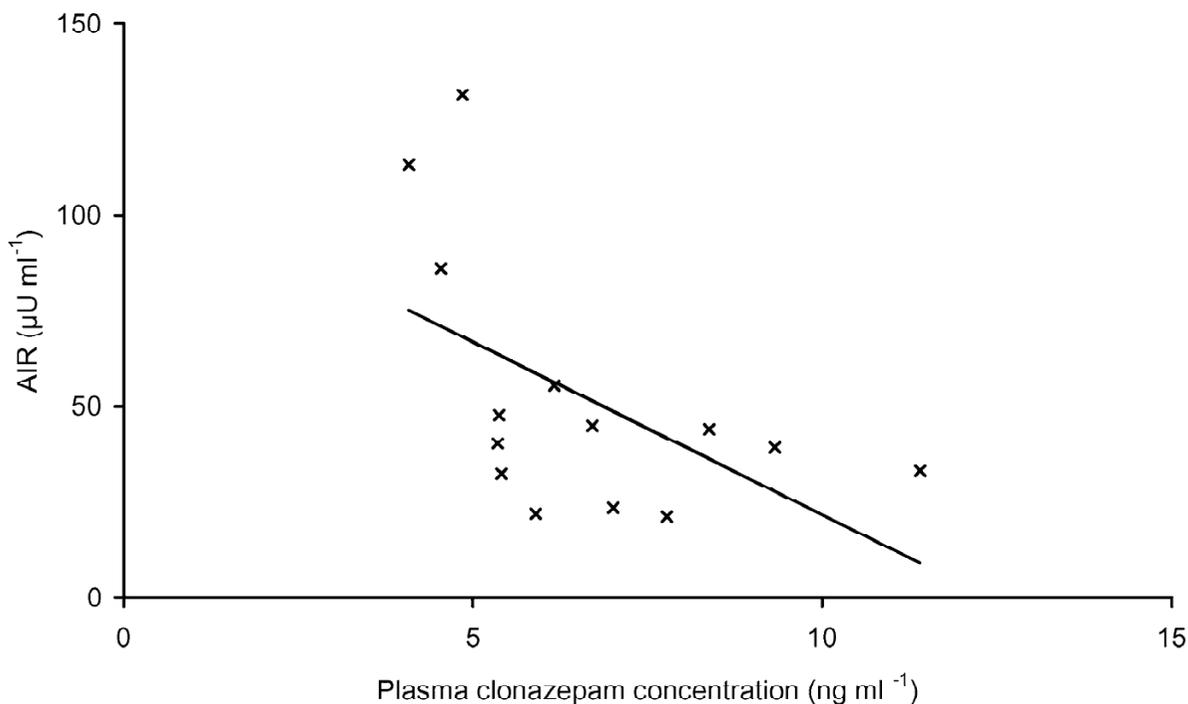
Figure 4
Correlation of acute insulin response (AIR), calculated from serum insulin concentrations obtained during the 10 first minutes after glucose injection, with the plasma concentrations of clonazepam obtained at time 0 min, just before glucose injection (r' = -0.609, P < 0.05, n = 14).

Table 1: Values of the parameters of insulin secretion, glucose tolerance, insulin sensitivity and glucose effectiveness according to the different treatment-groups.

Treatment	AIR (μUml^{-1})	K_g ($\times 10^{-2}min^{-1}$)	S_i ($\times 10^{-4}\mu Uml^{-1}min^{-1}$)	S_g ($\times 10^{-2}min^{-1}$)
Diazepam	52.9 ± 13.7	2.27 ± 0.26	3.52 ± 0.52	2.98 ± 0.30
Clonazepam	51.2 ± 8.6	1.78 ± 0.37	3.70 ± 0.79	2.39 ± 0.48
Placebo	53.9 ± 10.7	2.65 ± 0.31	3.50 ± 0.47	3.42 ± 0.37

observation may be the result of the hypoglycemia measured at that time (2.4 ± 0.1 mmoll^{-1}) and resulting from the insulin bolus, since acute hypoglycemia has been reported to affect brain function and activation [16]. On the other hand, the treatment by either benzodiazepine also induced a significant biological effect on plasma cortisol concentrations, which were decreased in the benzodiazepine-treated groups as compared with controls. This result is in accordance with previous studies in the literature [17,18], although not all studies have reported that a
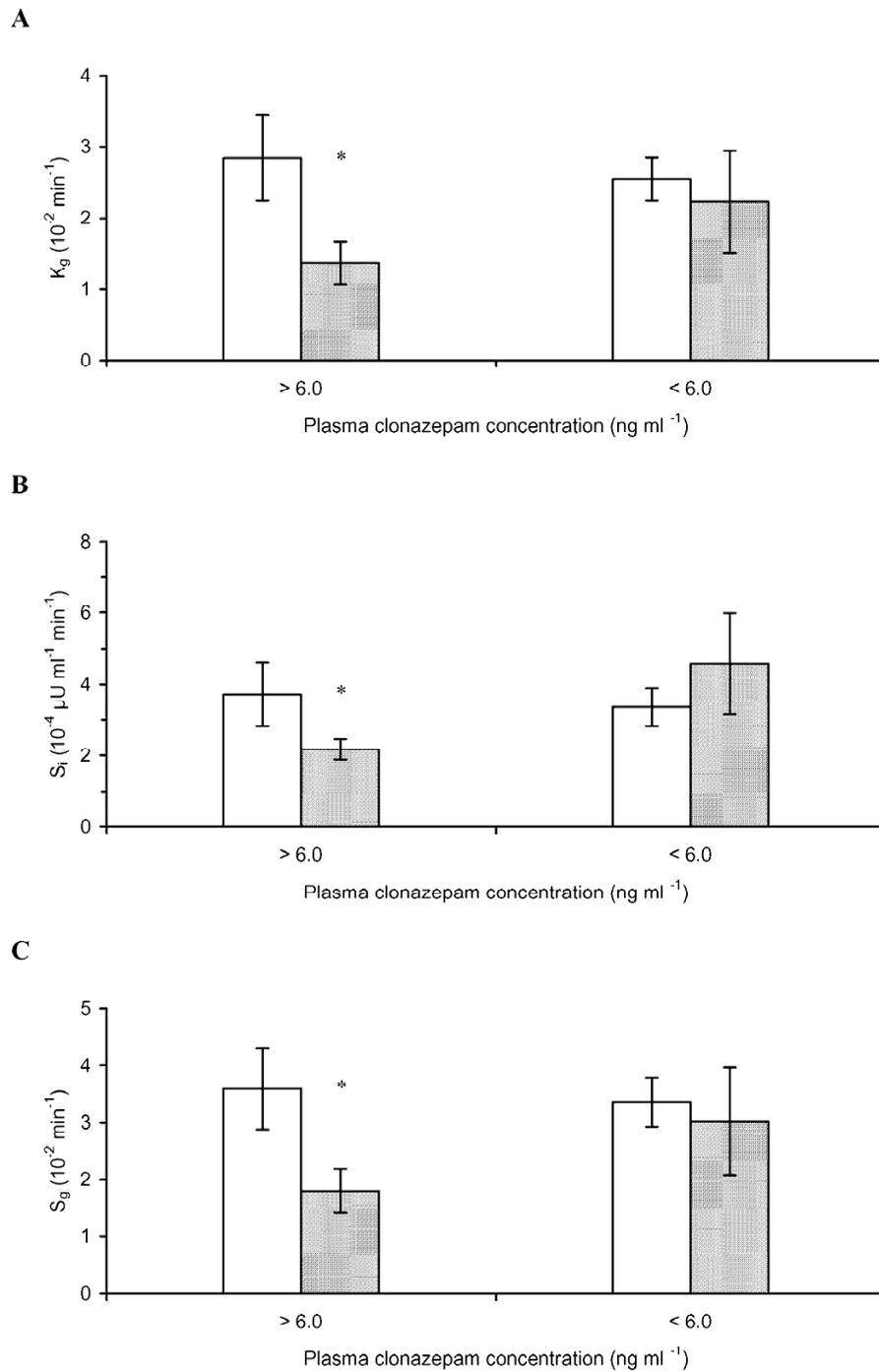
Figure 5
Effects of clonazepam on glucose tolerance, insulin sensitivity and glucose effectiveness. A Effect of clonazepam on glucose tolerance (K_g) according to plasma clonazepam concentration. *White bars*, controls; *Striped bars*, treated; * $P < 0.05$ *vs* placebo. B Effect of clonazepam on insulin sensitivity (S_i) according to plasma clonazepam concentration. *White bars*, controls; *Striped bars*, treated; * $P < 0.05$ *vs* placebo. C Effect of clonazepam on glucose effectiveness at basal insulin (S_g) according to plasma clonazepam concentration.*White bars*, controls; *Striped bars*, treated; * $P < 0.05$ *vs* placebo.

single dose of benzodiazepine affects cortisol secretion in healthy volunteers [19]. This effect of benzodiazepines on cortisol is in agreement with the inhibiting action of gabaergic agonists on the corticotropin releasing hormone secretion, as observed *in vitro* in rats [20]. It can also be observed that there is a relative increase in plasma cortisol concentrations following the lowest glucose concentrations (at time 70 minute), and that this cortisol response to insulin-induced hypoglycemia is not affected by either benzodiazepine, in accordance with some [21] but not all previously reported data [22].

Considering the metabolic effects, the present study shows that clonazepam is able to reduce the acute insulin response to a glucose challenge in healthy volunteers, in a concentration-dependent manner. This result is in accordance with previously reported data showing that Diazepam Binding Inhibitor (DBI), which is an endogenous ligand for both central and peripheral benzodiazepine receptors, dose-dependently inhibited glucose-induced insulin secretion *in vitro* in rat isolated islets [23,24]. In the same way, it supports previous observations that benzodiazepines or related compounds alter glucose tolerance *in vivo* in the rat [25] or in patients [4]. Other experiments however have shown *in vitro* that drugs acting specifically at peripheral benzodiazepine receptors inhibit glucose-induced insulin secretion [5] by reducing oxidative metabolism [26], whereas clonazepam, which binds very weakly to peripheral sites [27], is ineffective [5]. The peripheral-type receptor agonist, 4'-chlordiazepam, was also reported to increase glycaemia after intraperitoneal administration in the mice [28]. These discrepant data concerning clonazepam may be due to species variations or the result of an indirect central effect of the drug in our study. At the concentrations obtained with the single dose administered, there was no overall effect of clonazepam on the acute insulin response according to the different groups. Diazepam was ineffective as well, which may be the result of too low plasma concentrations over time. It is noteworthy to observe that both drugs however have significant psychotropic effects as well as effects on cortisol, even after 75 min after the end of the infusion, suggesting differential potencies relative to these effects.

On the other hand, this study shows for the first time that a benzodiazepine can reduce both insulin sensitivity (Si) and non-insulin-mediated glucose disposal (Sg and its components), provided that its concentration is above the admitted therapeutic threshold. Apart from the peak concentrations, this was especially the case for clonazepam but not for diazepam. There is no clear explanation for that observation. However, since insulin sensitivity and non-insulin mediated glucose disposal have been shown to increase even shortly after muscular

activity [13], it can be tentatively speculated that the well-known muscle relaxant action of the benzodiazepine may alter insulin sensitivity and glucose disposal.

Conclusions

Even if clinical metabolic events related to benzodiazepines are uncommon, the results of the present study on both insulin secretion and insulin action prompt to suggest that, on the long term, these drugs may be a potential risk factor for glycemic dysregulation.

Competing interests

None declared.

Author's contributions

HC participated in the design of the study, managed the research, wrote the standard operating procedures, handled data, conducted statistical analyses and participated in drafting the manuscript. IM collected informed consents, supervised selection procedures and clinical investigations, reported verified data on the CRF. NM participated in clinical tasks described above. BL carried out benzodiazepines assays. JFB computed glucose tolerance, insulin secretion and insulin sentivity indexes. PP conceived of the study and coordinated the data analysis and the draft writing. All authors read and approved the final manuscript.

Acknowledgements

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Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1472-6904/4/3/prepub