Bezafibrate in skeletal muscle fatty acid oxidation disorders

A randomized clinical trial

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ABSTRACT

Objective: To assess whether bezafibrate increases fatty acid oxidation (FAO) and lowers heart rate (HR) during exercise in patients with carnitine palmitoyltransferase (CPT) II and very long-chain acyl-CoA dehydrogenase (VLCAD) deficiencies.

Methods: This was a 3-month, randomized, double-blind, crossover study of bezafibrate in patients with CPT II (n = 5) and VLCAD (n = 5) deficiencies. Primary outcome measures were changes in FAO, measured with stable-isotope methodology and indirect calorimetry, and changes in HR during exercise.

Results: Bezafibrate lowered low-density lipoprotein, triglyceride, and free fatty acid concentrations; however, there were no changes in palmitate oxidation, FAO, or HR during exercise.

Conclusion: Bezafibrate does not improve clinical symptoms or FAO during exercise in patients with CPT II and VLCAD deficiencies. These findings indicate that previous in vitro studies suggesting a therapeutic potential for fibrates in disorders of FAO do not translate into clinically meaningful effects in vivo.

Classification of evidence: This study provides Class I evidence that bezafibrate 200 mg 3 times daily is ineffective in improving changes in FAO and HR during exercise in adults with CPT II and VLCAD deficiencies. **Neurology® 2014;82:607-613**

GLOSSARY

CI = confidence interval; **CPT** = carnitine palmitoyltransferase; **FAO** = fatty acid oxidation; **FFA** = free fatty acid; **HR** = heart rate; **LDL** = low-density lipoprotein; **MET** = metabolic equivalent; **PPAR** = peroxisome proliferator-activated receptor; **VLCAD** = very long-chain acyl-CoA dehydrogenase; **Vo2max** = maximal oxygen uptake capacity.

In patients with carnitine palmitoyltransferase (CPT) II and very long-chain acyl-CoA dehydrogenase (VLCAD) deficiencies, residual enzyme activity is high enough to ensure normal fatty acid oxidation (FAO) at rest.^{1,2} However, symptoms are provoked by exercise, because patients are unable to increase their FAO during exercise.^{1,2} Patients therefore have to rely to a greater extent on carbohydrate oxidation during exercise. As a result, muscle and liver glycogen stores are more rapidly depleted during exercise in these conditions, which can lead to energy crisis, muscle pain, and rhabdomyolysis.

A carbohydrate-rich diet can improve exercise tolerance³ but cannot fully compensate for the energy deficit during exercise in these patients. An attractive therapeutic approach could be to boost the residual enzyme activity. Peroxisome proliferator-activated receptor (PPAR) agonists may act in this way and have been studied in disorders of FAO.^{4,5} Two of the 3 PPAR isoforms, PPAR α and δ , are crucial for the regulation of processes involved in the uptake, transport, and oxidation of energy substrates.⁶ Bezafibrate is a pan-agonist that modifies *CPT2* and *VLCAD* gene expression mainly through the PPAR δ isoform.⁶⁻⁸ Dose-response experiments in muscle cells indicated a stimulation of FAO by bezafibrate concentrations ranging from 50 to 200 μ mol/L, which are typical levels obtained on therapy.^{9,10}

Supplemental data at
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In vitro studies on cells from patients with deficiencies of CPT II and VLCAD demonstrated that bezafibrate upregulates residual enzyme activity and protein levels via transcriptional regulation.^{4,5,11} Furthermore, an uncontrolled 6-month treatment study with 600 mg daily bezafibrate increased scores of life quality on the 36-Item Short Form Health Survey in 6 patients with CPT II deficiency.^{12,13}

We hypothesized that a clinical improvement due to bezafibrate in vivo would be associated with an increase in FAO during exercise and a better exercise tolerance in patients with disorders of FAO.

METHODS Design. The study was a randomized, placebo-controlled, double-blind, parallel, crossover trial.

Standard protocol approvals, registrations, and patient consents. The ethics committee of Copenhagen and the Danish Medicines Agency approved the study (EUdract number 2008-006704-27 plus amendment to include subjects from age 16 years; ClinicalTrials.gov: NCT00983788), and the study was monitored by the Good Clinical Practice Unit of Copenhagen University Hospital, Denmark. All subjects were informed orally and in writing about the study and gave written consent to participate.

Setting. The study was conducted at the Neuromuscular Research Unit, Rigshospitalet, University of Copenhagen, Denmark.

Patients. Inclusion criteria were patients with biochemically and genetically confirmed CPT II and VLCAD deficiencies, aged 16–70 years. Twelve patients were included between September 1, 2009 and August 31, 2011. Two patients were withdrawn (see results section). Thus, results are based on findings in 5 persons with CPT II deficiency (all males) and 5 with VLCAD deficiency (2 females and 3 males). One patient was heterozygous for a *CPT2* gene mutation. This patient was included based on results from an earlier study showing that heterozygous patients can be symptomatic, and that he specifically had a significantly lower FAO during exercise compared with healthy controls, and comparable to patients carrying 2 pathogenic *CPT2* gene mutations.¹ All patients, including the patient who was heterozygous for a mutation in the *CPT2* gene, had a typical history of exercise intolerance, rhabdomyolysis, and myoglobinuria. One patient had been treated with bezafibrate up until 4 weeks before inclusion in this study. One patient took a calcium antagonist for hypertension and methylphenidate for idiopathic hypersomnia. The female patients included were treated with oral contraceptives. Seven patients were French, and 3 were Danish. Exclusion criteria were competing disorders, kidney and liver disorders, allergy toward fibrates, pregnancy, and breastfeeding.

Randomization and blinding. A pharmacist not otherwise involved in the study dispensed active and placebo tablets according to a computer-generated randomization list. Investigators were supplied with letters describing treatment allocation for each subject number in a sealed envelope. Participants were randomly assigned following simple randomization procedures (computerized random numbers) to 1 of 2 treatment groups: either bezafibrate 200 mg 3 times daily PO followed by a treatment period with placebo, or vice versa. Bezafibrate and placebo tablets were identical in appearance. They were packed

in containers and consecutively numbered for each patient according to the randomization schedule stating either treatment period 1 or treatment period 2. Everyone, except the pharmacist, remained blinded to the treatment assignment.

Protocol. The study protocol consisted of 4 periods: 1) a 1-month observation period, 2) a 3-month treatment period, 3) a 2-month wash-out period, followed by 4) another 3-month treatment period. In the 4 periods, 5 cycle tests were performed: one incremental maximal exercise test in the 1-month observation period, and 4 constant workload cycle tests before and after each of the 2 treatment periods (figure e-1 on the *Neurology*[®] Web site at www.neurology.org).

One-month observation period. The subjects were instructed to eat a carbohydrate-rich diet for 3 days before the study. To determine a workload of 50% to 60% of maximal oxygen uptake capacity ($\dot{V}O_{2max}$) to be used for evaluating treatment responses in treatment periods, subjects performed an incremental cycle exercise test to exhaustion as described.¹⁴ There were no interim analyses. However, the following blood samples were used as safety parameters before and during treatment periods: creatine kinase, hepatic enzymes (aspartate aminotransferase, alanine aminotransferase, γ -glutamyltransferase, and alkaline phosphatase), bilirubin, creatinine, total cholesterol, high-density lipoprotein, low-density lipoprotein (LDL), and triglycerides.

Substrate turnover studies. Experiments began at 9 AM. To ensure the most optimal conditions for the subjects, breakfast was served 2 to 3 hours before exercise testing. Based on other studies, breakfast did not interfere with the measurements.^{1,2} Venous catheters were inserted in the cubital vein (stable-isotope infusion) and in the distal cephalic vein (blood sampling). A heating pad wrapped around the hand was used to arterialize the blood samples.¹⁵ A primed, constant rate infusion of [U - ^{13}C]-palmitate ($0.0026 \text{ mg kg}^{-1} \text{ min}^{-1}$, primed by a $0.085 \text{ mg kg}^{-1} \text{ NaH}^{13}\text{CO}_3$ bolus) was delivered by a Gemini PC2 pump (IMED, San Diego, CA). Two and a half hours after the start of the isotope infusion, subjects cycled to exhaustion or for 60 minutes at a workload of 50% to 60% of $\dot{V}O_{2max}$. Heart rate (HR) and the level of perceived exertion (Borg Scale)¹⁶ were monitored every other minute during exercise. Blood and expired gases were collected every 10 minutes, and preparation of tracers and tracer calculations were performed as described.¹⁴

Analyses of blood samples and expired $^{13}CO_2$. Venous blood was sampled in syringes containing $10 \mu\text{L}$ 0.33 M ethylenediaminetetraacetic acid/mL blood. The blood was immediately spun at 4°C and analyzed for lactate and glucose on a YSI model 2300 STAT plus (YSI Inc., Yellow Springs, OH). Insulin and isotope enrichments were analyzed as described.¹⁴ Free fatty acids (FFAs) were analyzed by photospectrometry (GloMax multiple plate-reader; Promega, Madison, WI). Acylcarnitine profiles at rest and during exercise were analyzed on and off treatment with bezafibrate (Waters Quattro Micro API, Milford, MA).

Primary outcome measures. The primary outcome measures were changes in FAO and HR during exercise. We expected that bezafibrate would induce a further 1.7-fold exercise-induced increase in palmitate oxidation (figure e-2), and hence lower the HR during exercise, which would indicate improved exercise tolerance.¹⁷ The measures would provide Class I evidence for the question of whether the effect of bezafibrate 200 mg 3 times daily could improve changes in FAO and HR during exercise in adults with CPT II and VLCAD deficiencies.

Secondary endpoints. Secondary endpoints included normalization of abnormal acylcarnitine concentrations, lowering of

perceived exertion during exercise, assessed by using a visual analog scale (Borg score), a reduction in episodes of myoglobinuria, and increased energy expenditure in METs (metabolic equivalents) assessed by Bouchard questionnaires and accelerometers (Sense Wear body monitoring system). The Bouchard questionnaire is a valid physical activity record in which the patients note their activities every 15 minutes during a 3-day period. An accelerometer measures physical activity and is worn as an armband on the upper arm for 3 consecutive days.¹⁸ Patients were instructed to wear it at all times and to remove it only during any water activity. The armband counted steps and calories burned.

Statistics. Values are presented as mean $\pm$ standard error. Differences between rest and exercise in the individual as well as between treatments were assessed by a paired Student *t* test and 95% confidence interval (CI). However, in those cases in which the normality test failed, a Wilcoxon signed-rank test was used. A *p* value <0.05 (2-tailed testing) was considered significant. SigmaPlot version 11 (Systat, San Jose, CA) was used to perform the statistical analyses. Based on the expectation that bezafibrate should increase palmitate oxidation 1.7-fold more than normal during exercise in these conditions, an 80% power to detect a possible significant difference (*p* = 0.05, 2-sided) between bezafibrate and placebo, 11 patients were required in the trial (figure e-2).

RESULTS Patients. Ten of 12 patients completed the study (figure 1 and table 1). Two patients withdrew, one during the observation period because of lack of motivation and one during the first treatment period because of a gallbladder stone. Thus, results are based on 10 patients. The results from primary and secondary endpoints did not differ between patients with CPT II and VLCAD deficiencies, and therefore data from the 2 conditions have been pooled. Baseline characteristics are presented in table 1.

Primary outcome measures. Fat oxidation. Palmitate oxidation and total FAO during exercise did not improve with bezafibrate in any of the patients compared with placebo (95% CI, total FAO: -4.7 to 5.1 and palmitate oxidation: -0.3 to 2.2) (figure 2). The exercise-induced increase in total fat and palmitate oxidation rates was only 2-fold and severely blunted compared with the normal 5-fold increase found in healthy subjects (figure 2).

Heart rate. In accordance with no effect of bezafibrate on fat oxidation, the HR response to exercise

Figure 1 Flow diagram for the bezafibrate study

Table 1 Baseline characteristics before first treatment period with either bezafibrate or placebo

	Bezafibrate	Placebo
Sex	4 M	4 M and 2 F
Age, y	38 ± 3 (31–46)	40 ± 8 (16–65)
BMI, kg/m ²	30 ± 1 (27–31)	25 ± 1 ^a (21–29)
CK, U/L	368 ± 173 (103–848)	5,511 ± 3,981 (182–25,300)
$\dot{V}O_{2\max}$	27 ± 3 (19–32)	28 ± 3 (17–38)

Abbreviations: BMI = body mass index; CK = creatine kinase; $\dot{V}O_{2\max}$ = maximal oxygen uptake capacity.

Values are count or mean ± standard error (range). The nominal difference in the CK value was driven by one severely increased CK value in one patient after a strenuous journey from Paris to Copenhagen.

^a $p < 0.05$ vs placebo group.

was identical on placebo and bezafibrate (95% CI, −9.1 to 6.9) (figure 3).

Secondary endpoints. Perceived exertion. Further evidence for a similar level of perceived exertion on

the 2 treatments was the identical Borg scores (95% CI, −1.3 to 1.7) (figure 3).

Acylcarnitine profiles. Acylcarnitine profiles were abnormal at rest and during exercise in all VLCAD patients (C14) and in 3 of 4 CPT II patients (C16, C18:2) (data missing from one CPT II patient). Treatment with bezafibrate did not change abnormal values at any sampling time.

However, carnitine increased significantly with bezafibrate treatment in both patients with CPT II and VLCAD deficiency (CPT II: 66 ± 6 vs 42 ± 4 $\mu\text{mol/L}$ [95% CI, −42.3 to −6.7], $p = 0.02$, and VLCAD: 53 ± 8 vs 27 ± 6 $\mu\text{mol/L}$ [95% CI, −49 to 4], $p = 0.01$).

Energy expenditure. We found no difference in METs day^{−1} with bezafibrate treatment vs placebo using the Bouchard questionnaire ($n = 6$, 163 ± 16 vs 168 ± 19 METs day^{−1} [95% CI, −2 to 13], $p = 0.1$). We also attempted to assess energy expenditure by using accelerometers; however, compliance

Figure 2 Fat metabolism

(A) Plasma palmitate concentration, (B) plasma free fatty acid (FFA) concentration, (C) palmitate oxidation, and (D) total fatty acid oxidation (FAO) at rest (gray) and during exercise at 50% to 60% of maximal oxygen uptake capacity ($\dot{V}O_{2\max}$) (black) without and with bezafibrate (green) in 10 patients with carnitine palmitoyltransferase II ($n = 5$) and very long-chain acyl-CoA dehydrogenase ($n = 5$) deficiencies. Values are mean ± standard error (SE). * $p < 0.05$ rest vs exercise; § $p < 0.05$ placebo vs bezafibrate. Dotted line indicates the mean fatty acid/palmitate oxidation at the 40th minute of exercise in 7 healthy controls exercising at 50% to 60% of $\dot{V}O_{2\max}$, and the shaded area is ±SE.

Figure 3 Exercise capacity

Perceived exertion (Borg) (A) and heart rate (B) during and at the end of exercise at 50% to 60% of maximal oxygen uptake capacity with placebo (closed circle) and bezafibrate (open circle) in 10 patients with carnitine palmitoyltransferase II ($n = 5$) and very long-chain acyl-CoA dehydrogenase ($n = 5$) deficiencies. Values are mean $\pm$ standard error.

was low, and data from both placebo and bezafibrate treatments could only be obtained in a paired manner from 3 patients (placebo: 142 ± 9 METs day⁻¹ vs bezafibrate: 152 ± 2 METs day⁻¹). This difference was considered insignificant (2 increased and 1 had a decrease in METs day⁻¹).

Episodes of myoglobinuria. One of the patients experienced an episode of rhabdomyolysis and myoglobinuria while having the flu. The episode occurred during bezafibrate treatment. The patients reported no other attacks of myoglobinuria.

Substrate concentrations and turnover. Plasma palmitate and total FFA increased in both studies with exercise (95% CI, palmitate: 17 to 251, and FFA: -951 to 470) ($p < 0.05$; figure 2). Bezafibrate treatment lowered plasma palmitate and total FFA concentrations at rest and during exercise ($p < 0.05$; figure 2). In accordance with the similar levels of fat combustion during exercise on and off bezafibrate, respiratory exchange rate was similar at rest and increased similarly during exercise in both groups (table 2). These

data are supported by the increase in the total carbohydrate oxidation, which did not differ between treatment groups either (table 2).

$\dot{V}O_2$ max, workload, and exercise duration. $\dot{V}O_2$ max was 28 ± 2 mL O₂ kg⁻¹ min⁻¹ ($n = 9$). Patients exercised at a constant workload of 49 ± 9 W during all cycle trials. The duration of exercise, which could be sustained, did not differ significantly between treatments (placebo: 44 ± 5 minutes vs bezafibrate: 47 ± 4 minutes).

Plasma metabolites and hormones. Plasma lactate increased and glucose decreased similarly on placebo and bezafibrate (table 2). Insulin decreased to the same extent during exercise on both treatments (table 2).

Adherence to treatment. After each treatment period, collected remaining tablets showed a drug compliance of 89% on both treatments. Adherence to the treatment was also inferred by the lowering of FFAs in plasma (figure 2) and lowering of other lipid profiles. Bezafibrate lowered serum triglycerides (placebo: 1.5 ± 0.2 mmol/L vs bezafibrate: 1.1 ± 0.1 mmol/L, $p < 0.01$) and serum LDL (placebo: 3.4 ± 0.3 mmol/L vs bezafibrate: 3.0 ± 0.3 mmol/L, $p = 0.04$, $n = 9$) and tended to increase serum high-density lipoprotein (placebo: 1.1 ± 0.9 mmol/L vs bezafibrate: 1.2 ± 0.1 mmol/L, $p = 0.088$, $n = 9$) and decrease serum cholesterol (placebo: 5.2 ± 0.4 mmol/L vs bezafibrate: 4.7 ± 0.3 mmol/L, $p = 0.065$).

No adverse events. There were no adverse events recorded. One patient was hospitalized with an episode of myoglobinuria and rhabdomyolysis based on viral infection, but this episode was not considered as an adverse event by the investigators.

DISCUSSION The main findings of this study are that 1) bezafibrate does not induce increased FAO during exercise in patients with CPT II and VLCAD deficiencies, 2) exercise tolerance, indicated by HR and rates of perceived exertion, does not improve with bezafibrate, 3) bezafibrate lowers available FFAs in plasma, which has a negative influence on fat oxidation, 4) abnormal acylcarnitine profiles are not modified by bezafibrate, and 5) activity levels of the patients, based on a Bouchard questionnaire and data from accelerometer measurements, did not increase during bezafibrate treatment.

Assessing efficacy of potential treatments for rare diseases remains a challenge. One major reason is the difficulty in recruiting large cohorts of patients to reach sufficient power. Another important issue is the necessity to define clinically meaningful endpoints, and relevant biomarkers. In contrast to muscular dystrophies, metabolic myopathies such as CPT II and VLCAD

Table 2 Substrate kinetics, indirect calorimetry, and plasma metabolites at rest and during exercise with placebo and bezafibrate

	Placebo		Bezafibrate	
	Rest	End exercise	Rest	End exercise
$Rd_{\text{palmitate}}$, $\mu\text{mol kg}^{-1} \text{min}^{-1}$	2.2 ± 0.4	3.8 ± 0.8^a	1.6 ± 0.3	2.4 ± 0.3^a
$Ra_{\text{palmitate}}$, $\mu\text{mol kg}^{-1} \text{min}^{-1}$	2.2 ± 0.4	4.0 ± 0.9^a	1.7 ± 0.3	2.4 ± 0.3^a
RER	0.83 ± 0.02	0.92 ± 0.02^a	0.82 ± 0.02	0.93 ± 0.02^a
Total CHO, mmol min^{-1}	1.1 ± 0.2	7.2 ± 0.7^a	1.1 ± 0.2	8.2 ± 0.8^a
Insulin, pmol L^{-1}	56 ± 10	28 ± 5^a	95 ± 22	31 ± 7^a
Glucose, mmol L^{-1}	5.3 ± 0.2	4.7 ± 0.2^a	5.4 ± 0.1	4.9 ± 0.3
Lactate, mmol L^{-1}	1.1 ± 0.1	3.4 ± 0.6^a	1.2 ± 0.1	3.7 ± 1.1^a

Abbreviations: CHO = carbohydrate oxidation; $Ra_{\text{palmitate}}$ = palmitate production (rate of appearance of palmitate); $Rd_{\text{palmitate}}$ = palmitate utilization (rate of disappearance of palmitate); RER = respiratory exchange rate ($\dot{V}O_2/\dot{V}CO_2$).

Values are mean $\pm$ standard error.

^a $p < 0.05$ rest vs exercise.

deficiencies, do not present with fixed muscle weakness, but are characterized by intermittent, exercise-related symptoms of muscle pain and rhabdomyolysis. Thus, current muscle strength assessments used in clinical trials for muscular dystrophies are not clinically meaningful in metabolic myopathies. Subjective quality-of-life scores, such as the 36-Item Short Form Health Survey, can only be used as secondary outcome measures, because of the absence of specific “scoring” items of muscle symptoms in this survey. Therefore, tests under conditions that provoke symptoms, i.e., during bouts of dynamic exercise, are better suited to disclose treatment effects in metabolic myopathies. Another attractive possibility for assessment in these myopathies is to directly measure the primary defect that is causing symptoms during exercise, namely, the fat oxidation rate. Oxidation of fat can be measured in vivo by use of the stable-isotope technique during exercise.

Based on evidence of an in vitro effect of bezafibrate in FAO disorders, and subjective improvements of life quality in an uncontrolled study of 6 patients with CPT II deficiency,¹² we designed a randomized, double-blind, placebo-controlled trial in order to study the effect of bezafibrate on FAO in patients with CPT II and VLCAD deficiencies. Despite the promising findings of a bezafibrate-induced increase in palmitoyl L-carnitine oxidation levels by up to 284% in cultured fibroblasts from patients with CPT II deficiency,¹² our findings show that this in vitro efficacy measure does not translate into a clinically meaningful effect in vivo. We predicted that the patients should at least increase palmitate oxidation 1.7-fold more than normal, based on previous assessments of FAO in healthy subjects and patients compound heterozygous or heterozygous for *CPT2* and *VLCAD* gene mutations.^{1,2} However, FAO was unaffected by bezafibrate, which was consistent in all

10 patients we studied. The lack of effect could potentially be explained by counteracting mechanisms in vivo, which are not active in vitro. One such potential mechanism is the bezafibrate-induced reduction in substrate availability for FAO. Thus, bezafibrate significantly lowered the plasma concentrations of palmitate and other FFAs. Because the level of FAO is known to be directly related to the plasma concentration of FFA,¹⁹ bezafibrate could have a negative influence on FAO rates. However, lowering of FFA could also limit the potential toxic effect of accumulated long-chain fatty acids in patients with VLCAD deficiency, and to some extent CPT II deficiency, thus decreasing the episodes of rhabdomyolysis. However, the only episode of myoglobinuria during this trial occurred in a patient receiving bezafibrate treatment.

A limitation of this study was the lack of success 1) to encourage the patients to contact their local doctors and arrange to have their safety parameters taken, and 2) to collect results of the safety parameters. Furthermore, a limitation of technically complicated studies in rare patients, such as the present study, is the low number of patients one can enroll. However, the results of this study are convincing because findings were very uniform in all subjects, and consistently showed no effect of bezafibrate on the primary endpoints in each patient. The lack of effect of bezafibrate was not caused by low adherence to the drug protocol, because compliance to the trial was universally high, which was also supported by the bezafibrate-induced effects on lipid profiles. In line with the lowering of plasma FFAs, bezafibrate lowered triglycerides and LDL as described in other studies.²⁰

Fibrates have so far been prescribed for the treatment of hyperlipidemia in conjunction with improvement of glucose tolerance.²¹ Recent molecular and cellular studies indicate additional effects mediated through the activation of PPARs or PPAR- γ coactivator-1 α receptors of bezafibrate and fenofibrate on several other metabolic diseases, besides disorders of FAO. These include adipose triglyceride lipase deficiency, mitochondrial myopathies, and Huntington disease.^{21–24}

Our findings suggest that these in vitro results, which point to a therapeutic potential of fibrates, should be interpreted with caution and should be followed up by in vivo studies using clinically meaningful endpoints.

AUTHOR CONTRIBUTIONS

Mette Cathrine Ørngreen, MD, contributed to conception and design of the study, acquisition, analysis and interpretation of data, did the statistical analysis and drafted, revised, and approved the article. Karen Lindhardt Madsen, BSc, Nicolai Preisler, MD, and Grete Andersen, MD, contributed to acquisition and interpretation of data, and revised and approved the article. John Vissing, MD, and Pascal Laforêt, MD,

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