Acta Neuropsychiatrica

cambridge.org/neu

Effects of erythropoietin on body composition and fat–glucose metabolism in patients with affective disorders

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Original Article

Cite this article: Vinberg M, Højman P, Pedersen BK, Kessing LV, Miskowiak KW. (2018) Effects of erythropoietin on body composition and fat–glucose metabolism in patients with affective disorders. *Acta Neuropsychiatrica* 30:342–349. doi: 10.1017/neu.2018.16

Received: 20 November 2017
Revised: 24 April 2018
Accepted: 30 April 2018
First published online: 8 June 2018

Key words:
body composition; cognition; erythropoietin; glucose; mood disorders

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Abstract

Background: Erythropoietin (EPO) has been suggested to improve metabolism and also cognition, but human studies are scarce. This randomised controlled trial aimed to investigate whether EPO treatment influences body composition and fat and glycated haemoglobin (HbA1c) and fasting glucose, and whether these changes would be associated with previous observed cognitive benefits of EPO. *Method:* In total, 84 non-obese patients with treatment-resistant unipolar depression or bipolar disorder in remission were randomised to 8 weekly EPO (40,000 IU) or saline (NaCl 0.9%) infusions in a double-blind, parallel-group design. Patients underwent dual X-ray absorptiometry scans at baseline and week 14 (6 weeks after treatment completion). Cognitive measures were assessed and fasting levels of cholesterol, lipoprotein fractions, triacylglycerides, glucose and HbA1c were obtained at baseline, week 9 and follow-up week 14. *Results:* In total, 79 patients had complete pre- and post-treatment data (EPO: $N = 40$, saline: $N = 39$). EPO had no cumulative effect on body composition and markers of fat metabolism. The EPO-treated group exhibited significantly lower HbA1c levels after 8 weeks treatment [$F(1, 80) = 8.51$, $p = 0.005$], however, 6 weeks after treatment termination a significantly higher fasting glucose levels [$F(1, 79) = 5.85$, $p = 0.02$] and HbA1c levels [$F(1, 79) = 5.85$, $p = 0.02$] were seen. The latter increase in HbA1c was further significantly correlated with a better cognitive outcome on verbal memory ($r = 0.25$, $p = 0.03$). *Conclusion:* Repeated EPO infusions had no cumulative effect on body composition in this cohort of patients with affective disorders, however, EPO modulated HbA1c and fasting glucose and this was associated with patients' improvement of verbal memory.

Significant outcomes

- Repeated erythropoietin (EPO) infusions induced changes in glycated haemoglobin (HbA1c) and fasting glucose.
- These changes were associated with improvement of verbal memory.
- Repeated EPO infusions had no cumulative effect on body composition and fat metabolism.

Limitations

- The study included physically healthy non-obese participants, only.
- All participant except two received psychotropic medication.
- The moderate sample size.

Introduction

EPO is a candidate treatment for cognitive impairment and mood symptoms in patients with mood disorders (1,2). EPO is a 165-amino-acid protein and member of the cytokine superfamily with structural similarity to growth hormone (3,4). It is mainly produced in the kidneys, liver, uterus but also in peripheral endothelial cells, muscle cells as well as in the central nervous system (CNS) by neurons and astrocytes (5). In particular, the later points to a role of EPO as a potent growth factor that may protect CNS cells against apoptosis and promote proliferation of neuronal cells (6). Although the canonical activity of EPO is to

regulate red blood cell production in the bone marrow, EPO is also capable of improving cardiac function, reducing fatigue, increasing neuroplasticity and improving cognitive function in affective disorders (6,7). Further, preclinical studies indicate that EPO reduces blood glucose levels, improves glucose tolerance and attenuates diet-induced weight gain (8,9). These findings are complemented by preliminary clinical evidence that EPO improves glycaemic control in patients with diabetes (10).

Cognitive deficits are prevalent in patients with metabolic disorders and in patients with affective disorders (e.g. diabetes mellitus obesity and metabolic syndrome) (11,12). Further, patients with affective disorders exhibit a reduced lifespan (13) which has been suggested to be partly due to abnormal metabolic and glycaemic pathways in patients with severe mental disorders (14). Therefore, treatment strategies that can effectively treat both cognitive deficits and metabolic abnormalities represent possible integrated treatment avenues to improve overall functioning in patients with affective disorders (11,12). Although EPO has promising effects on metabolism, studies of clinical samples are scare. The potential effects of EPO on metabolism in clinical studies therefore warrant further investigation.

The present study of the effects of EPO on metabolic parameters is based on our two parallel randomised, placebo-controlled, double-blind studies of 8 weeks of weekly EPO versus saline treatment of patients with treatment-resistant depression (TRD) and bipolar disorder (BD). These trials suggested that EPO treatment had beneficial effects on cognitive function in TRD and BD (15). Notably, these effects persisted beyond the acute treatment phase after red blood normalisation, suggesting that they were partially independent of red blood cell changes. The present study of the effects of EPO on metabolic parameters is based on blood samples and dual X-ray absorptiometry (DXA) scans from these two identical parallel trials in partially remitted BD patients and TRD patients, respectively, which are described in details in the original study protocol (16).

Aims of the study

Specifically, we aimed to clarify (i) whether EPO treatment influences body composition as well as fat and glucose metabolism in patients with affective disorders and (ii) whether such treatment-related metabolic changes correlated with the cognitive improvement in EPO treated patients. We hypothesised that (i) EPO would have beneficial effects on the body composition and on fat and glucose metabolism and (ii) these changes would be associated with the observed cognitive benefits of EPO.

Methods and materials

Study design and participants

The two original EPO trials from which the present data are derived had a double-blind, placebo-controlled, parallel-group design and their primary outcomes are published elsewhere (16–18) and a detailed description on the full study design, power calculations and all predefined measured can be find in Miskowiak et al. (16). Patients were recruited through the Copenhagen Clinic for Affective Disorders, Psychiatric Centre Copenhagen. Eligible patients had an ICD-10 diagnosis of TRD, defined as failure to respond to adequate treatment with at least two different types of antidepressants given in adequate time and doses (19) with moderate depression Hamilton Depression Rating Scale 17 items (20) (HDRS-17 score ≥17) (sub-study 1) or a ICD-10 diagnosis of BD in full or partial remission [HDRS-17 and Young Mania Rating Scale (YMRS) scores ≤14] (21) with moderate cognitive difficulties according to the Cognitive and Physical Functioning Questionnaire (22) (score ≥4 on ≥2 domains) (sub-study 2). For an extensive description of the screening procedure, randomisation and masking, exclusion criteria, and safety precautions see Miskowiak et al. (17, 18). Written informed consent was obtained from all patients. The study was approved by the Capital Regions Local Ethics Committee: H-C--2008-092, Danish Medicines Agency 2612-4020, EudraCT: 2008-04857-14, Danish Data Agency: 2008-41-2711 and ClinicalTrials.gov: NCT 00916552.

Procedures

Patients were randomised to receive weekly infusions of EPO (Eprex; 40 000 IU/ml; Janssen-Cilag, Birkerød, Denmark) or saline (NaCl 0.9%) for 8 weeks in addition to their current medication. Patients underwent DXA scans at weeks 1 (baseline) and 14 (6 weeks after treatment completion). Blood tests were taken at a weekly basis for safety monitoring. Additional blood samples for examination of biomarkers were taken at weeks 1, 9 (after treatment completion) and 6 weeks after at week 14. At these time points, mood symptoms were also rated at these time points with the HDRS-17 and the YMRS. Finally, cognitive function was assessed at weeks 1, 9 and 14 with a comprehensive neuropsychological test battery.

Sampling and biochemical analyses

Routine blood samples including haemoglobin (Hgb), fasting plasma glucose, plasma levels of cholesterol content of lipoprotein fractions and triacylglycerides (TAG), and HbA1c were obtained were obtained at weeks 1, 9 and 14 by venepuncture in the fasting state between 8:00–10:00 a.m.

Body composition and fat-free mass

Fat and fat-free mass for the whole body, trunk and extremities were measured using DXA scanning (Lunar Prodigy Advance; GE Medical Systems Lunar, Milwaukee, WI, USA). DXA scanning does not distinguish between subcutaneous and intra-abdominal fat located in the trunk region. Software (Prodigy, encore 2004, version 8.8; GE Lunar Corp., Madison, WI, USA) was used to estimate regional and total fat mass and fat-free tissue. Patients were scanned at baseline and at follow-up week 14.

Neurocognitive tests

The included neurocognitive test battery covered the domains of attention, memory and executive function, encompassing Rey Auditory Verbal Learning Test (RAVLT) (23) total recall across trials I–V, the total score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Coding (24), verbal fluency test with the letter D, WAIS-III Letter-Number Sequencing (25), Trail Making Test B (26) and the time to correct responses on the Rapid Visual Information Processing from Cambridge Cognition (CANTAB). As previous reported in Miskowiak et al. (17) these neuropsychological tests comprising a measure of 'speed of complex cognitive processing' were improved by EPO versus saline in BD. A composite scores of 'speed of complex cognitive processing' based on the six neuropsychological tests was created; for full details see Miskowiak et al. (18).

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Statistical analysis

The primary measure of interest in the present was change in body fat distribution from baseline to the post-treatment follow-up (weeks 1–14) as defined a priori in the original study protocol (16). The secondary measures of interest were changes in blood levels of lipid and glucose from baseline to weeks 9 (1 week post-treatment) and 14 (6 weeks follow-up). Independent samples *t*-tests or χ^2 tests were used when appropriate to compare the clinical and demographic variables between the two treatment groups (EPO, saline) at baseline, week 9 and at follow-up week 14. Effects size, reflected by Cohen's *d*, was calculated for statistically significant between-group differences.

All comparative analyses between the treatment groups were intention-to-treat using last observation carried forward for missing values in accordance with the *a priori* defined strategy (16). Data were analysed using repeated measures analyses of covariance (ANCOVA) with treatment group (EPO, saline) as the between-group factor, time (weeks 1–9, weeks 1–14) as the within-subject factor and with adjustment for stratification variables (age and gender) to minimise effects of any baseline imbalances. The *F*-test (*F*) of significance was used to assess the effects of the covariate(s) and time. Spearman's correlations were used to analyse bivariate associations between change over time (from weeks 1–9 and weeks 1–14) and changes in fasting glucose, HbA1c and RAVLT, and the Cognitive Composite scores, respectively. Finally, due to the explorative nature of the present analyses Bonferroni corrections were not included.

Results

Study characteristics

Patients were included and randomised for the two trials between September 2009 and October 2012. Of the 84 randomised patients, one patient withdrew on the inclusion day, leading to inclusion of 83 patients (EPO: $N = 41$; saline: $N = 42$) of whom 74 completed per protocol (PP) (EPO: $N = 34$; saline: $N = 40$) (Fig. 1). Of the nine patients who did not complete PP, six patients (EPO) discontinued medication after five to six infusions because of increased platelet count ($>4 \times 10^9$/l) but completed all assessments and three patients (one EPO week 6, one EPO week 10, one placebo, week 10) were admitted to the hospital because of acute suicide risk. As seen from Table 1, the two groups were well balanced in terms of baseline characteristics ($p \geq 0.12$). All patients continued their medication as usual and did not change their medication in the study period.

The effects of EPO on body composition

Total body, fat and muscle mass as well as total fat-free mass at baseline and at week 14 are presented in Table 1. At baseline, EPO

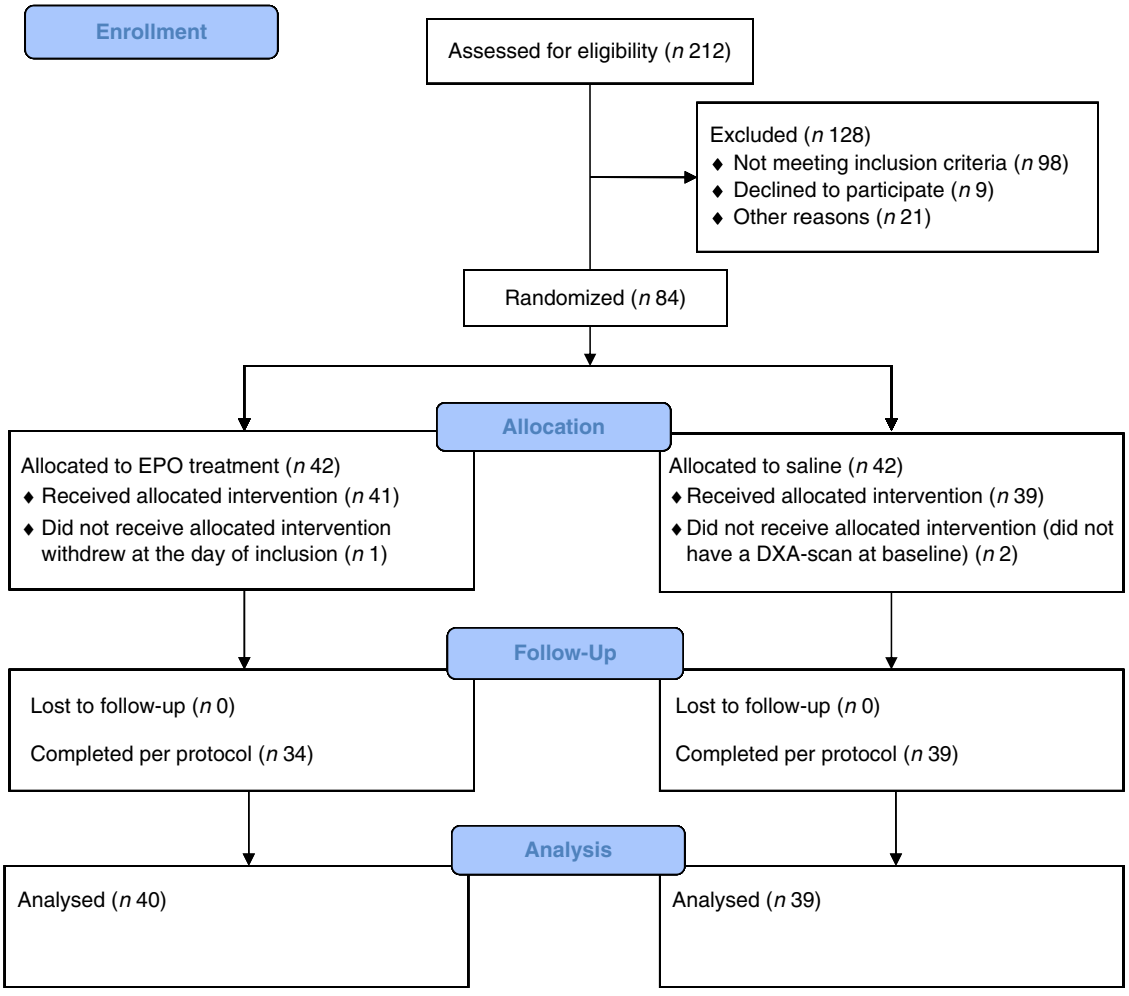

Fig. 1. CONSORT flow diagram. EPO, erythropoietin; DXA, dual X-ray absorptiometry.

https://doi.org/10.1017/neu.2018.16 Published online by Cambridge University Press

Table 1. Patients ($N = 80$) with affective disorders, characteristics, body mass index (BMI), metabolic markers and body composition

	Baseline			Week 9			Follow up week 14		
	EPO group ($N = 41$)	Saline group ($N = 39$)	p	EPO group ($N = 40$)	Saline group ($N = 39$)	p	EPO group ($N = 40$)	Saline group ($N = 39$)	p
Age (years)	40 (10)	43 (13)	0.13						
Gender [no. female (%)]	13 (72)	14 (66)	0.47						
Years of education	15 (3)	15 (3)	0.64						
BMI (kg/m^2)	25 (3)	24 (3)	0.07				24 (3)	24 (3)	
RAVLT	45 (10)	46 (9)	0.47	50 (9)	47 (11)	0.31	51 (10)	49 (11)	0.27
HDRS-17	14 (6)	14 (6)	0.96	11 (7)	12 (7)	0.20	11 (6)	12 (7)	0.18
Metabolic regulation									
Hgb (mmol/l)	8.4 (0.75)	8.6 (0.74)	0.99	9.2 (1.0)	8.5 (0.8)	0.001	8.5 (0.9)	8.5 (0.9)	0.52
p-HDL (mmol/l)	1.6 (0.4)	1.6 (0.5)	1.00	1.6 (0.5)	1.6 (0.5)	0.75	1.6 (0.4)	1.6 (0.4)	0.75
p-LDL (mmol/l)	3.0 (0.79)	3.0 (0.9)	0.95	3.0 (0.7)	2.9 (0.9)	0.78	2.9 (0.6)	3.0 (1.0)	0.73
p-TAG (mmol/l)	1.2 (0.5)	1.2 (0.6)	0.96	1.2 (0.5)	1.2 (0.7)	0.76	1.1 (0.6)	1.1 (0.6)	0.84
p-glucose (mmol/l)	4.9 (0.6)	4.9 (0.6)	0.73	4.9 (0.5)	4.8 (0.4)	0.31	5.1 (0.7)	4.8 (0.5)	0.05**
HbA1c (%)	5.3 (0.3)	5.3 (0.3)	0.78	5.1 (0.3)	5.3 (0.3)	0.02*	5.5 (0.3)	5.3 (0.3)	0.002***
Body composition (kg)									
Total mass	74.8 (12.8)	74.8 (13.1)	0.55				73.3 (12.1)	74.0 (13.0)	0.58
Total fat mass	33.8 (11.7)	33.3 (10.1)	0.70				32.5 (7.9)	32.6 (10.5)	0.78
Total muscle mass	47.9 (12.4)	46.9 (10.6)	0.56				48.5 (10.7)	47.7 (10.7)	0.40
Total fat-free mass	51.5 (11.0)	49.6 (10.8)	0.33				51.3 (11.0)	50.5 (11.0)	0.40

EPO, erythropoietin; HbA1c, glycated haemoglobin A1c; HDRS-17, Hamilton Depression Rating Scale 17 items; Hgb, Haemoglobin; RAVLT, Rey Auditory Verbal Learning Test; p-glucose, fasting plasma glucose; p-HDL, plasma high-density lipoprotein; p-LDL, plasma low-density lipoprotein; p-TAG, plasma triacylglycerides.
Mean standard deviation in brackets.
*Cohen's $d = 0.91$, **Cohen's $d = -0.33$, ***Cohen´s $d = -0.76$.

and saline groups showed no differences in body composition total mass, total fat mass, total muscle mass and total fat-free mass (p-values >0.33). Repeated-measures ANCOVA including age and sex as covariates comparing body composition in the EPO versus saline treated patients from weeks 1 to 14, F (1,78) revealed no effect of EPO versus saline on total body mass, fat mass, muscle mass and total fat-free mass (p-values ≥0.10).

The effects of EPO on lipid status

Content of lipoprotein fractions high-density lipoprotein (HDL), low-density lipoprotein (LDL) and TAG at baseline, at week 9 and at follow-up week 14 are presented in Table 1. There were no significant differences in these variables between the EPO and saline groups at baseline (p-values ≥0.16). Repeated measures ANCOVA including age and sex as covariates, revealed no effects of EPO versus saline (p ≥0.49) or any general changes over time in HDL, LDL and TAG (p ≥0.08) (Table 2).

The effects of EPO on fasting glucose and HbA1c levels

Fasting glucose levels and HbA1c at baseline, week 9 and week 14 are presented in Table 1 and also illustrated in Fig. 2. At baseline, EPO and saline groups were comparable for fasting glucose ($p = 0.86$) and HbA1c ($p = 0.93$). Repeated measures ANCOVA including age and sex as covariates revealed no significant effects of EPO versus saline on fasting glucose levels from baseline to week 9 ($p = 0.28$) (see Table 2). However, repeated measures ANCOVA revealed a significant increase in fasting glucose levels in the EPO versus saline treated patients from baseline to week 14 [$F(1, 79) = 5.85$, $p = 0.02$] (increase, mean ± SD: EPO 0.2 ± 0.76 mmol/l; saline: −0.16 ± 0.66 mmol/l).

Repeated measures ANCOVA including age and sex as covariates revealed a significant effect of EPO versus saline on HbA1c change from baseline to week 9 (Table 2). Specifically, the EPO group exhibited a significant *decrease* in HbA1c levels in the treatment period [$F(1, 79) = 8.51$, $p = 0.005$] (decrease, mean ± SD: EPO 0.15 ± 0.27; saline: −0.01 ± 0.14). Repeated measures ANCOVA also revealed a significant effect of EPO versus saline from baseline to week 14. However, at follow-up, week 14 the EPO group, this time, exhibited a significantly *increase* in HbA1c levels from baseline to week 14 [$F(1, 79) = 5.85$, $p = 0.02$] (increase, mean ± SD: EPO 0.28 ± 0.34; saline: −0.01 ± 0.17).

Exploratory analyses removing the nine patients who did not complete PP from the analyses the results did not change the results. Further, as eight of the non-completers had to withdraw from the study after 5–7 weeks due to haemopoitic side effects a further exploratory analyses was conducted adding HbA1c levels from week 5 (where, the eight participants from the EPO group still received EPO) and these analyses did not influence the results (results not presented).

Post-hoc analysis revealing a possible indirect association between the EPO induced increase in Hgb levels and HbA1c levels

As Hgb levels were increased in response to EPO versus saline treatment at week 9 [$F(1, 81) = 7.64$, $p = 0.01$] an additional *post-hoc* repeated measures ANCOVA were conducted including changes in Hgb levels (from baseline to week 9 = delta Hgb) as an additional covariate. First, when looking at the treatment period from baseline to week 9, adding the change in Hgb levels, the effect of treatment group was still significant [$F(1, 79) = 4.2$, $p = 0.04$] and the decrease in HbA1c showed a non-significant an association with the increase in Hgb level (delta Hgb) in the EPO group [$F(1, 79) = 3.0$, $p = 0.09$]. Repeating the same ANCOVA concerning the time period from baseline to follow-up week 14, the effect of group on HbA1c levels was no longer significant and there was a borderline significant effect of EPO on delta Hgb [$F(1, 74) = 3.6$, $p = 0.06$]. Thus, the EPO induced change in Hgb levels may indirectly be associated with the present EPO induced changes in HbA1c levels.

Table 2. Markers of metabolic regulation

	Row 1, time main effects (weeks 1, 9) (p-values) Row 2, time main effect by treatment group (weeks 1, 9) (p-values)	Row 1, time main effects (weeks 1–14) (p-values) Row 2, time main effect by treatment group (weeks 1, 14) (p-values)
p-HDL	$p = 0.08$, $F = 0.31$	$p = 0.78$, $F = 0.08$
	$p = 0.49$, $F = 0.49$	$p = 0.80$, $F = 0.09$
p-LDL	$p = 0.12$, $F = 0.24$	$p = 0.56$, $F = 0.34$
	$p = 0.75$, $F = 0.10$	$p = 0.98$, $F = 0.001$
p-TAG	$p = 0.11$, $F = 2.61$	$p = 0.76$, $F = 0.10$
	$p = 0.79$, $F = 0.79$	$p = 0.55$, $F = 0.35$
p-Glucose	$p = 0.56$, $F = 0.34$	$p = 0.09$, $F = 2.91$
	$p = 0.28$, $F = 1.18$	$p = 0.02$, $F = 5.85$
HbA1c	$p = 0.69$, $F = 0.16$	$p = 0.09$, $F = 2.92$
	$p = 0.005$, $F = 8.51$	$p = 0.02$, $F = 5.85$

EPO, erythropoietin; HbA1c, glycated haemoglobin A1c; p-glucose, fasting plasma glucose; p-HDL, plasma high-density lipoprotein; p-LDL, plasma low-density lipoprotein; p-TAG, plasma triacylglycerides.

Results for all patients with affective disorders ($N = 80$) (EPO group $N = 41$, saline group $N = 39$), df (1, 80).

At baseline (week 1) upon treatment completion (week 9) and follow-up (week 14). The F-test = F values of significance was used to assess the effects of the covariate(s) and time, Factor time F, and p values (main effect of time), and factor time by treatment group interaction and p values (main effect of treatment group). Covariates for repeated-measures analyses of covariance in all analyses: age and gender.

https://doi.org/10.1017/neu.2018.16 Published online by Cambridge University Press

Correlation between fasting glucose, HbA1c, body composition and cognition, and whether cognition scores any association with changes in HbA1c levels

Finally, in further analyses on cognition: Spearman's correlations were used to analyse bivariate correlations between change over time (from weeks 1–9 and weeks 1–14) in RAVLT scores and cognitive composite score and fasting glucose, HbA1c. There were no significant correlations between changes in the treatment period (baseline to week 9) in fasting glucose and HbA1c and cognitions score (RAVLT and the cognitive composite scores) ($p \geq 0.07$). However, as illustrated in Fig. 3, the increase in HbA1c at follow-up was significantly correlated with a more improvement over time in RAVLT score at follow-up ($r = 0.25$, $p = 0.03$) but not with the change in fasting glucose ($r = 0.19$, $p = 0.10$). Changes in composite cognitive scores at follow-up revealed no significant correlations with fasting glucose or HbA1c. There were no significant correlations between body composition measured as total body-, fat- and muscle- mass and changes in cognitive scores (weeks 1–14) ($p \geq 0.14$). Finally, in further post hoc repeated measures ANCOVA including age and sex as covariates adding our primary outcome parameter changes in verbal memory (total RAVLT scores) between weeks 1–14 and changes in the cognitive composite scores as covariates, respectively revealed no significant associations.

Discussion

The present study is the first to explore the effect of EPO on body composition, lipid and glucose status in patients with affective disorders. In contrast with our hypothesis 8 weeks weekly treatment with EPO revealed no significant effects on neither body composition or on lipid metabolism. However in accordance with our a priori hypothesis, 8 weekly infusions of high-dose EPO was associated with a significant decrease in HbA1c levels and improved HbA1c and fasting glucose. This was accompanied with a subsequent increase in fasting glucose and HbA1c levels 6 weeks after treatment completion, which seemed to be associated with the haemopoietic effects of EPO. Finally, the previously described beneficial effect of EPO on verbal memory correlated significantly with changes in HbA1c but seemed unrelated to changes in fat metabolism or body composition.

First, EPO reduced HbA1c levels in accordance with our hypothesis supporting the previous mentioned studies showing some evidence of glycaemic control improvement in patients with diabetes (10). At follow-up after 6 weeks, this decrease was inverted and instead a significant increase in fasting glucose and HbA1c levels were seen in the EPO group, which makes these results puzzling to interpret. However, the EPO-associated increase in HbA1c levels at follow-up may represent systemic glycemic control in the preceding 8–12 weeks. Since erythrocytes are freely permeable to glucose, the rate of formation of HbA1c is directly proportional to the ambient glucose concentration in which the erythrocyte circulates to the duration of the exposure and the turnover of the erythrocytes (27). Hence, EPO treatment may induce an elevation of the peripheral glucose level due to a

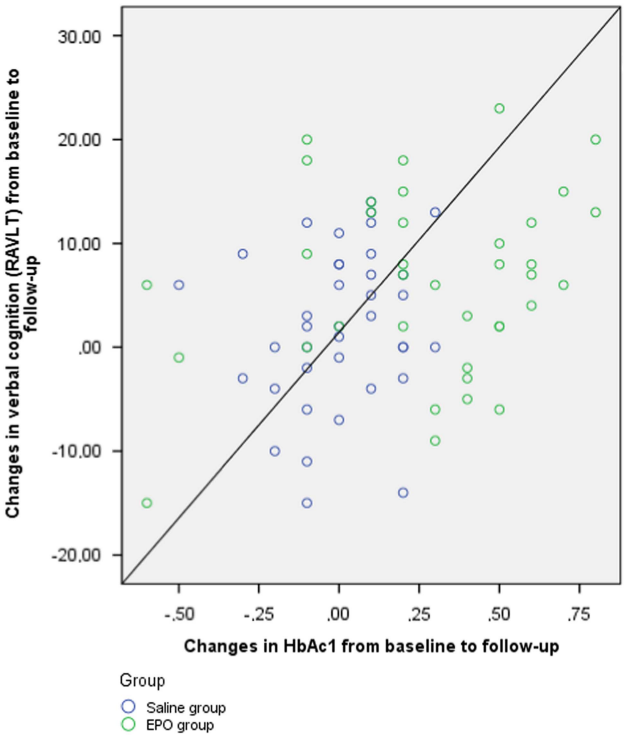

Fig. 3. Scatter plot of Spearman's correlations between change over time from baseline to follow-up week 14 for the erythropoietin (EPO) and the saline/placebo group in glycated haemoglobin A1c (HbA1c) levels and verbal cognition as measured with Rey Auditory Verbal Learning TEST (RAVLT).

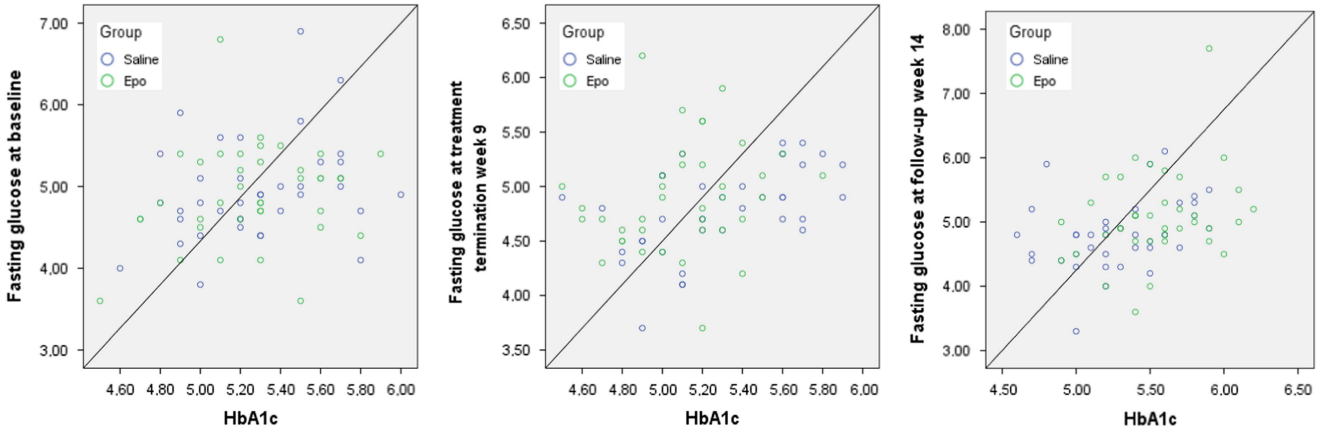

Fig. 2. Scatterplots of glycated haemoglobin A1c (HbA1c) and fasting plasma glucose levels for the erythropoietin (EPO) and the saline/placebo group at baseline, week 9 and week 14.

https://doi.org/10.1017/neu.2018.16 Published online by Cambridge University Press

higher turnover of the erythrocytes. This effect was reflected over time and could partly contribute to the elevated HgAc1 levels at follow-up. However, HbA1c was lower in the treatment period from baseline to week 9, so the increase at follow-up could also represent a compensatory effect, thus the found increase at follow-up may present a rebound effect after completion of the EPO treatment.

The change in fasting glucose level and increased HbA1c levels in the EPO-treated group at follow-up may be due to a well-functioning adapting mechanism in this physically healthy non-smoking and non-obese cohort of patients with affective disorders. This in turn may also explain the correlation between higher HgAc1 and a better outcome on RAVLT at follow-up as the brain may benefit from the larger capacity in glucose transport. However, EPO may regulate lipid and glucose metabolism differently in, for example, obese or in individuals with diabetes. The increase seen in Hg1Ac levels at follow-up is not necessarily due to any kind of dysregulation rather the increased peripheral glucose accessibility may have beneficial effects on brain metabolism. Nevertheless the effect of repeated infusion with EPO seems to influence HbA1c and fasting glucose over time and it would be of interest to investigate whether the effect of EPO treatment is mediated through an effect on the peripheral insulin and/or glucose tolerance.

The present study have several limitations: the study included physically healthy non-obese participants due to the strict exclusion criteria for our trial participant due to the safety profile of EPO. Accordingly, the failure to demonstrate any effects of EPO on body composition and fat metabolism, which contrast with preclinical findings, could be due to the participants' relatively normal fat metabolism. Therefore it cannot be excluded that EPO may modulate lipid, glucose metabolism differently in, for example, obese or in individuals with diabetes. Similarly, the demonstration of EPO associated decrease in HbA1c levels followed by an increase in HbA1c levels in this physical healthy cohort of patients may not apply to patients with serious somatic diseases. Further, we did not include extended analyses on glucose metabolism by calculating the Homeostasis Model Assessment (HOMA) a HOMA index that estimates steady state β cell function and insulin sensitivity as percentages of a normal reference population or by performing an oral glucose tolerance test in this sample of non-diabetic subjects. Finally, due to the exploratory nature of the study, we did not adjust the findings for multiple comparisons. The findings should therefore be consider merely hypothesis-generating and require replication.

We did add the covariates age and sex in all analyses and did not find any significant associations this may be due to the limited sample size and further the present study cohort had an over-representation of women (reflecting the well-known gender differences in the prevalence of depression). All the included patients, except two, did receive medical treatment. Further based on our previous studies from this cohort and the use of a randomised design there were no statistically significant differences in types of medication, number of prescribed medication or doses between the EPO treated and the saline group (17,18). Still, the possibility that medication could have influenced the present results in an unpredictable way should be considered. Further, other factors that can influence metabolic status such as diet and physical activity were not incorporated as covariates in the statistical models. However, due to RCT design and successful double-blinding it is assumed that these habits were equal between the in the two groups. Albeit these limitations EPO shows promising effects on metabolism and could be added to the list of potential pharmacological candidates (28) targeting the interface between mood disorders and the metabolic pathways. Although, we found a beneficial effect of EPO on HbA1c and fasting glucose in this rather small sample, the finding requires replication in larger cohorts of patients and physically and mentally healthy matched control groups. This finding and the possible beneficial association to cognition warrant further investigation in larger clinical studies including patients with different psychiatric and physical diagnoses.

Acknowledgement. The authors thank the study nurses, Susanne Sander and Hanne Nikolajsen and radiologist Anne Præstegaard for their great work with logistical planning, running the trial, and taking care of many practical issues related to the trial and the clinical staff at the Mood Disorder Clinic, Psychiatric Center Copenhagen, Rigshospitalet for valuable clinical support and help.

Authors' contribution. K.M., M.V. and L.V.K. conceived and designed the randomised controlled trial and P.H. and B.K.P. contributed to the conception and design of the present study. M.V. and K.M. recruited the patients and runned the study together with L.V.K. M.V. undertook the statistical analyses and wrote the first manuscript draft. All authors contributed to the writing and have approved the current version of the manuscript.

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