Erythropoietin activates classical brown adipose tissue through the erythropoietin receptor/ STAT3 pathway, improving obesity and glucose homeostasis in high fat diet-induced obese mice

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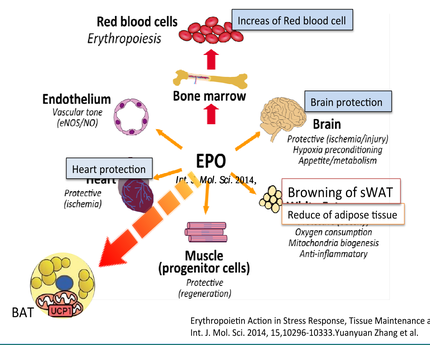
Objective

- Previous reports demonstrated that erythropoietin (EPO) induces body weight loss and improves insulin resistance in obese mice. As a part of its mechanisms, the browning of white adipose tissue (WAT) induced by high dose EPO treatment (3,000 IU/kg) contributes the improvement of obesity.

- The aim of this study is to elucidate the mechanism of thermogenesis in the classic brown adipose tissue (classical BAT, cBAT) .



Erythropoietin Action in Stress Response, Tissue Maintenance and Metabolism
Int. J. Mol. Sci. 2014, 15,10296-10333.Yuanyuan Zhang et al.

Materials and Methods

- Four-week-old male C57BL/6J mice were fed a high fat diet (HFD) and injected with recombinant human (rh)EPO. EPO (rhEPO 200 IU/kg, Epoetin-α, JCR Pharma, Japan) was administered three times per week for 4 weeks by intraperitoneal injection .

- Oxygen consumption (VO2) was measured to estimate the metabolic rate. And also, surface temperature on the interscapular BAT (iBAT) was used to quantify heat generation a thermal imaging camera .

- Metabolic parameters were measured at 8 weeks.

- Intraperitoneal glucose tolerance tests (IPGTT) (glucose, 1 g/kg) were performed in the overnight-fasted mice. And measurements of the levels of blood glucose and serum insulin.

- We also analyzed the expression of genes and proteins related to thermogenesis and that asosiated signal pathway on iBAT (By the methods of quantitative realtime PCR and western blot analysis).

Discussion

- We have investigated that EPO treatment significantly reduced the body weight and improved glucose intolerance in the HFD mice. WAT mass volume was decreased and iBAT mass volume was increased by the effect of EPO treatment.
  Katz O.*J Endocrinology* 2010
  Meng R . *PloS one* 2013
  Woo M. *Diabetes* 2014.

- Our study identified that the mRNA expression and protein of UCP1 were increased in the HFD group in comparison with the control group. Furthermore, the expression of UCP1 protein was upregulated with EPO treatment under the HFD condition. We considered that EPO treatment could have synergistic effect under the condition such as the adrenalin stimulation. The biological markers for BAT-specific differentiation (PRDM16, PPARα, PPAR γ and PGC1α,) were also increased in the interscapular BAT (cBAT).

- We confirmed that classical BAT expresses EPO receptor (EpoR). EPO has a direct effect through EPOR/STAT3 signaling pathway on the regulation of UCP1 in cBAT and related to differentiation pathway that MyomiR-133 regulates brown fat differentiation through Prdm16. Further investigation is required for these signals in detail by EPO.
  Li W et al, *Diabetes* 2013
  Sally Y et al, *Diabetologia* 2014
  Ruifeng T et al, *Nature commun* 2011

Conclusion

- EPO treatment, even at a lower dosage, activates heat production and lipolysis in cBAT through EPOR/STAT3 and MEF2-miR133-PRDM16 under HFD conditions. This mechanism leads to improve obesity and insulin resistance.

We have nothing to disclose.

Results

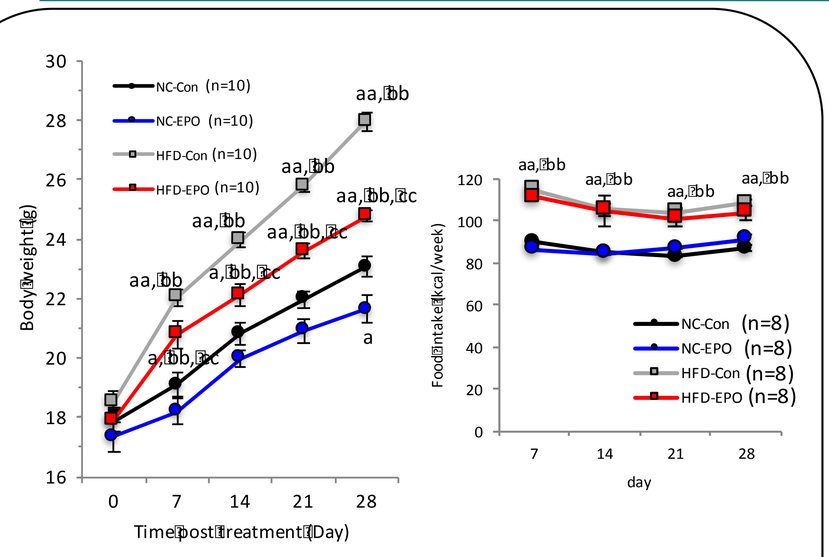


Fig1. EPO decreased body weight gain despite a lack of differences in food energy intake in dietary-induced obese mice. Values are means ± SE for 9-10 mice. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

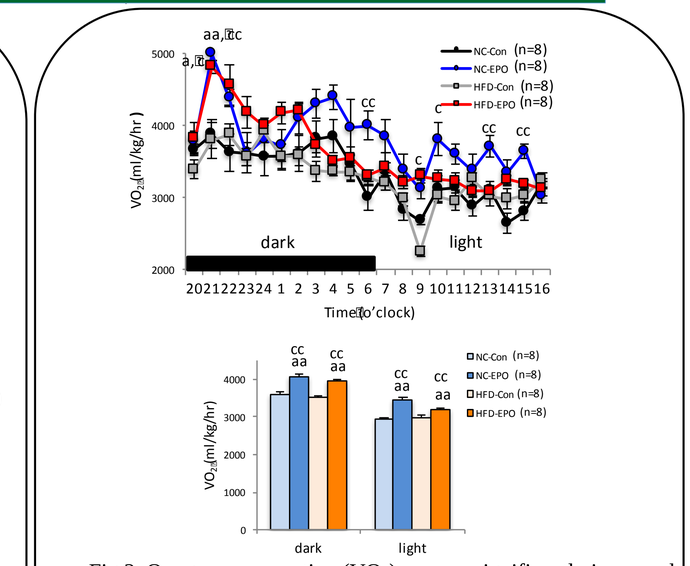


Fig 2. Oxygen consumption (VO_2), an was significantly increased in NC-EPO and HFD-EPO mice compared to NC-Con and HFD-Con mice in both the dark and light phases. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

	n	NC+Con	NC+EPO	HFD+Con	HFD+EPO
Body weight (g)	10	23±0.3	21.6±0.5 ^{a}	28±0.3 $^{aa, bb}$	24.8±0.2 $^{aa, bb, cc}$
Interscapular BAT mass (g)	9-14	0.06±0.002	0.11±0.007 $^{aa, cc}$	0.07±0.003 ^{bb}	0.13±0.006 $^{aa, cc}$
Subcutaneous WAT mass (g)	5	0.21±0.014	0.22±0.023	0.89±0.067 $^{aa, bb}$	0.7±0.051 $^{aa, bb, c}$
Epididiymal WAT mass (g)	9-15	0.24±0.03	0.23±0.024	0.85±0.04 $^{aa, bb}$	0.61±0.054 $^{aa, bb, cc}$
Glucose (mg/dl)	7-9	75.4±9.5	51.6±9.6	90.8±8.9 ^{b}	61.2±6.9
Total cholesterol (mg/dl)	4-8	83.5±2.3	86.8±2.9	146.3±4.9 $^{aa, bb}$	125.5±3.8 $^{aa, bb, cc}$
Triglyceride (mg/dl)	4-8	52.3±12.7	62±18.1	37.8±7.1 $^{a, b}$	45±8.8 ^{a}
Insulin (μU/ml)	7-9	12.4±2.1	9.1±1.1	13.6±1.6	10.6±1.0
HOMA-IR	7-9	2.3±0.46	1.1±0.23	3.1±0.59 ^{b}	1.5±0.18 ^{c}

Table 1.Glucose, Total cholesterol and HOMA-IR value were significantly elevated in HF mice and significantly lowered by EPO. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

Fig3. EPO improves glucose intolerance and insulin resistance.
A: The glucose tolerance. B: The insulin tolerance. Values are means ± SE for 7-9 mice. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

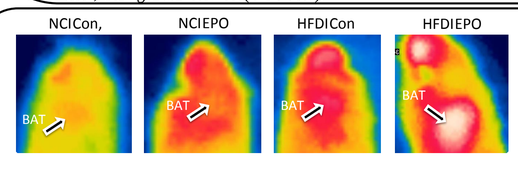


Fig 4. Interscapular BAT (iBAT) temperature in HFD-EPO mice (33.6 ± 0.8° C) was significantly higher than that in HFD-Con mice (32.5 ± 0.5° C) ($p < 0.05$) and NC-EPO mice (31.7 ± 0.6° C) was significantly higher than the temperatures in NC-Con mice (30.7 ± 0.5° C) ($p < 0.05$).

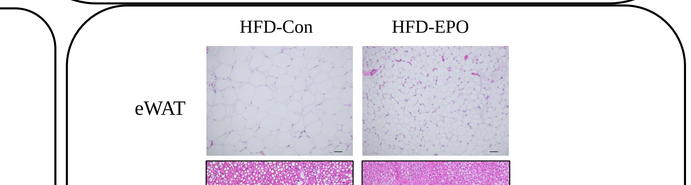


Fig 5. Histology of iBAT and epididymal WAT (eWAT) (HE staining). EPO change the composition of adipocytes and the number of lipid droplets of histology.

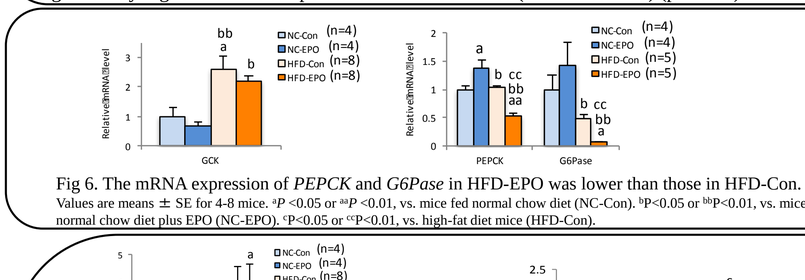


Fig 6. The mRNA expression of *PEPCK* and *G6Pase* in HFD-EPO was lower than those in HFD-Con.
Values are means ± SE for 4-8 mice. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

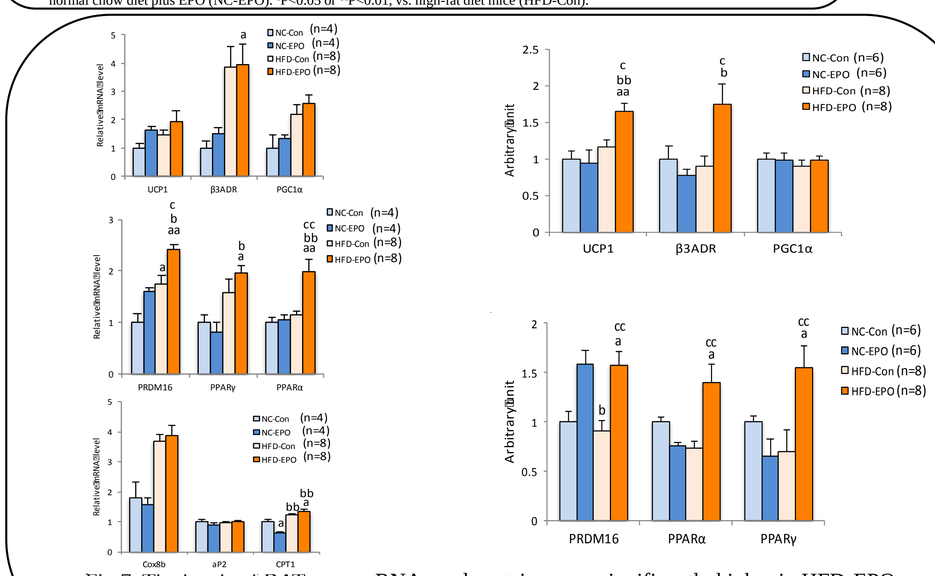


Fig 7. The levels of BAT gene mRNAs and proteins were significantly higher in HFD-EPO mice as compared with HFD-Con mice. Values are means ± SE for 4-8 mice. ^{a}P <0.05 or ^{aa}P <0.01, vs. mice fed normal chow diet (NC-Con). ^{b}P<0.05 or ^{bb}P<0.01, vs. mice fed normal chow diet plus EPO (NC-EPO). ^{c}P<0.05 or ^{cc}P<0.01, vs. high-fat diet mice (HFD-Con).

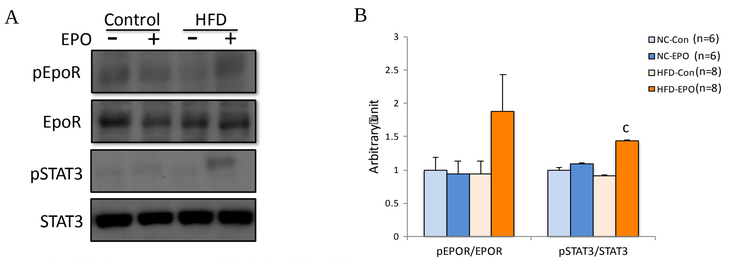


Figure 9. EPO activates EPOR-STAT3 signaling
A: Western blot analysis in iBAT. B: Relative protein levels. Values are mean ± SE for 6–8 mice. $^{c}P < 0.05$ or $^{cc}P < 0.01$, vs. high-fat diet mice (HFD-Con).

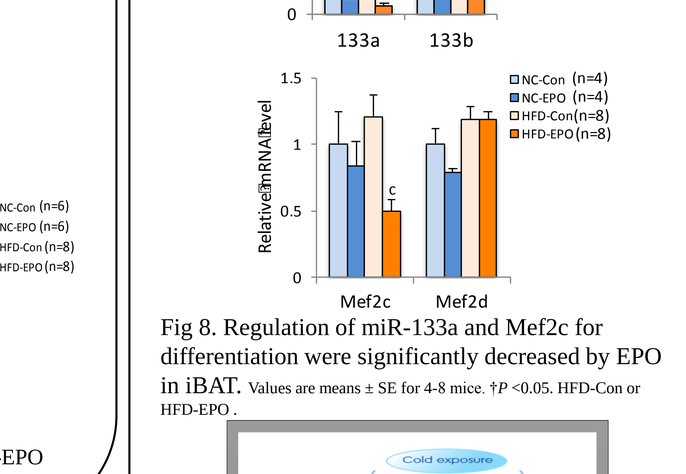
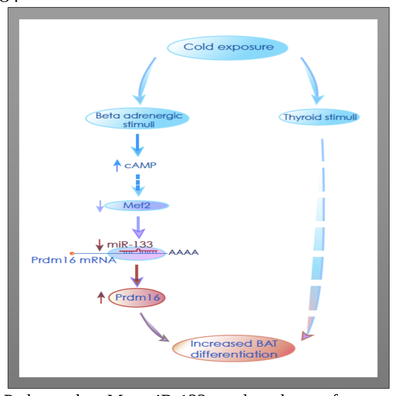


Fig 8. Regulation of miR-133a and Mef2c for differentiation were significantly decreased by EPO in iBAT. Values are means ± SE for 4-8 mice. †P <0.05. HFD-Con or HFD-EPO .



Pathway that MyomiR-133 regulates brown fat differentiation through Prdm16
Mirko T et al, NATURE CELL BIOLOGY 2012

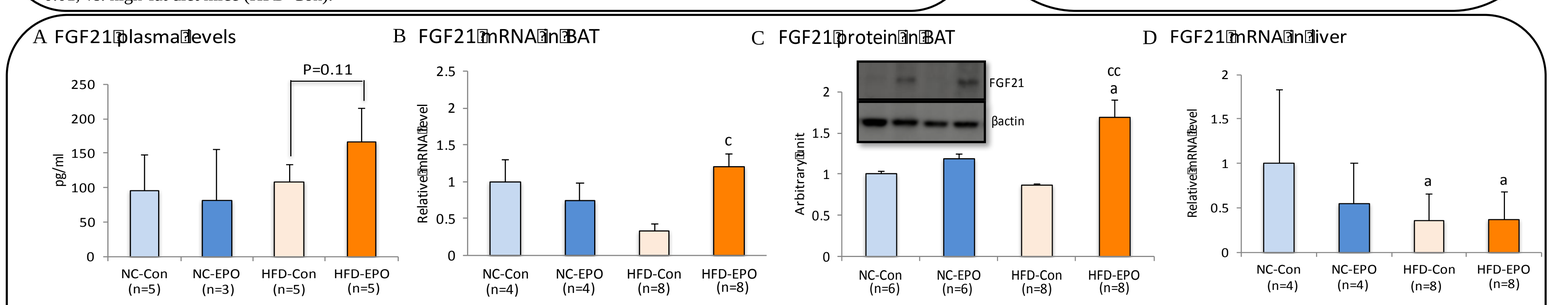


Figure 10. EPO promotes secretion of FGF21 from BAT and improves insulin resistance
A: FGF21 plasma levels. (n = 3–5). P = 0.11, vs. high-fat diet mice (HFD-Con). B: mRNA levels of FGF21 in Liver. (n = 4–8). C: mRNA levels of FGF21 in iBAT. (n = 4–8).
D: FGF21 protein level in iBAT (n = 6–8). $^{a}P < 0.05$ or $^{aa}P < 0.01$, vs. mice fed normal chow diet (NC-Con). $^{c}P < 0.05$ or $^{cc}P < 0.01$, vs. high-fat diet mice (HFD-Con).

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