mRNA was very low compared with the level of *FABP4* mRNA.

We also did *in vitro* experiments on human adipocytes. Levels of mRNA were measured using real-time PCR and the human glycerol kinase primers described by Guan *et al.*[1] Expression of mRNA was normalized for 18S RNA. Glycerol kinase mRNA expression in isolated human adipocytes was very low compared with the levels found in human kidney and mouse brown adipose tissue, two sites with high glycerol kinase activity (**Fig. 1e**). To assess the effect of rosiglitazone on gene expression, differentiated human adipocytes from primary cultures were treated for 48 h with 1 mol/l rosiglitazone. In contrast to the results by Guan *et al.*[1], we found no increase in glycerol kinase mRNA expression ($P = 0.79$; **Fig. 1f**). As observed *in vivo*, *FABP4* mRNA was increased by rosiglitazone ($P = 0.02$).

If glycerol kinase were induced, glycerol would be recycled in adipose tissue, resulting in a decreased glycerol output from adipose tissue. Rather than a decrease, we observed a nonsignificant increase in adipose tissue glycerol output in humans *in vivo*, suggesting that glycerol is not reused in adipose tissue after rosiglitazone treatment in humans.

The presence of a low glycerol kinase activity in adipose tissue has been well documented[3]. We observed a low level of glycerol kinase mRNA expression in both human adipose tissue and human adipocytes. Guan *et al.* presented data as relative inductions of glycerol kinase activity[1]. The threefold increase they described may still be negligible in absolute terms. Our findings of a lack of induction of glycerol kinase mRNA expression in response to rosiglitazone in human adipose tissue and cultured human adipocytes show that more work needs to be done in this area before accepting any relationship of glycerol kinase to TZD-induced insulin sensitization.

Human studies using TZDs show small falls in NEFA concentrations in the range of 8–30% (refs. 4–6), not always statistically significant, as was true in the present study. In contrast, most rodent studies show a decrease in NEFA concentration of 50–90% in response to TZD treatment[7,8]. Accordingly, the conflicting data on glycerol kinase induction by rosiglitazone between rodents and humans is consistent with the species-specific changes in NEFA concentrations by TZDs.

We are aware that depot-specific changes in adipose tissue in response to TZD treatment have been described. Guan *et al.* proposed that glycerol kinase induction lowers NEFA release from adipose tissue, and that this might account for the insulin-sensitizing action of TZDs[1]. However, the contribution of visceral adipose tissue to circulating NEFA concentrations is only ~7% (refs. 9,10), with the overwhelming contribution (74–78%) to circulating NEFAs being from the upper body subcutaneous adipose depot[9,10], the tissue studied here.

Because we studied patients after 12 weeks of treatment, it could be that we have missed early changes in glycerol kinase expression and that we are studying compensatory changes to the insulin sensitization. It seems difficult to argue, however, that any process that is fundamental to insulin sensitization could be switched off at a time when insulin sensitization is so clearly observed.

In summary, a 'futile metabolic cycle' involving glycerol is not induced in adipose tissue in humans treated with rosiglitazone, and thus does not contribute to the metabolic actions of TZDs in humans.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the Nature Medicine website for details).

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Guan *et al.* reply:

We are pleased that our report of TZD induction of adipose glycerol kinase[1] has prompted further investigation by other groups. Tordjman *et al.* recently confirmed our observation that rosiglitazone markedly induces glycerol kinase in mouse adipocytes[11]. In their correspondence, Tan *et al.* did not find increased glycerol kinase expression in rosiglitazone-treated human adipocytes or in adipose samples from rosiglitazone-treated type 2 diabetics. It is important to note that in the patients studied by Tan *et al.*, rosiglitazone treatment did not lower plasma levels of NEFAs. This differs from nearly all rodent models, as well as several controlled human trials reporting significant lowering of NEFA levels by rosiglitazone and other TZDs[5,12,13]. Human responses are likely to be more heterogeneous than those of inbred rodent models. If, as we have hypothesized, adipose glycerol kinase induction is one factor contributing to NEFA lowering by TZDs, then lack of glycerol kinase induction may not be surprising in patient populations in which NEFA levels do not respond to TZDs. Larger studies will be needed to determine the extent to which TZD induction of adipose glycerol kinase is variable in humans, and whether this correlates with reduction in NEFA levels upon TZD treatment.

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