The Sum of all Browning in FGF21 therapeutics

Paul Lee[1,3], Michael M Swarbrick[2] and Jerry R Greenfield[1,3]

Diabetes and Metabolism Division, Garvan Institute of Medical Research[1], Centre for Diabetes, Obesity and Endocrinology, Westmead Millennium Institute[2] and Department of Endocrinology, St Vincent's Hospital[3], Sydney, Australia

Correspondence author: Dr Paul Lee, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW, Australia 2010

Tel: 61 2 9295 8416 Fax: 61 2 9295 8481 Email: p.lee@garvan.org.au

Summary

FGF21-mimetics are a promising therapeutic tool that is believed to exert its anti-obesity effect partly through browning of white fat. Veniant *et al*. (2015) and Samms *et al*. (2015) present evidence arguing against fat browning to be the primary mechanism causal to weight loss following FGF21-based treatment in mice.

Fibroblast growth factor 21 (FGF21) is a master regulator of substrate turnover and orchestrates crosstalk between liver, white adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscle and pancreas. Pharmacologic FGF21 reverses obesity, diabetes, dyslipidaemia and hepatic steatosis in rodents and monkeys, seeding hopes that FGF21-based therapy could become a powerful weapon to combat metabolic syndrome. As FGF21 is a potent inducer of uncoupling protein 1 (UCP1) in WAT, WAT browning, the transformation of WAT into an energy-consuming BAT-like tissue - termed brite/beige fat, has been proposed to underlie FGF21-mediated metabolic benefits. Two recent publications, one in *Cell Metabolism* (Veniant et al., 2015), the other in *Cell Reports* (Samms et al., 2015), dissect the inter-dependence between WAT browning and therapeutic actions of FGF21-based treatment and conclude that the pharmacological effects of FGF21 are independent from WAT browning.

Both groups followed the same overall experimental paradigm and first compared interscapular BAT (iBAT) and inguinal WAT (igWAT) in mice at 21°C and/or thermoneutrality before and after FGF21 treatment, either with a long acting FGF21 analogue (FGF21-Fc) (Veniant et al., 2015) or hFGF21 infusion (Samms et al., 2015). As BAT is not required at thermoneutrality, iBAT showed expected signs of BAT whitening in thermoneutral mice. Following FGF21 treatment, UCP1 was upregulated in iBAT of 21°C-housed mice and whitened-iBAT of thermoneutral mice regained a uniform BAT phenotype. Significant igWAT browning with UCP1 protein induction was only observed in mice at 21°C. Despite differences in igWAT browning extent, FGF21 treatment however induced similar increase in energy expenditure without altering food intake, leading to similar weight loss and glycaemic/lipid improvement

at both temperatures. The authors then repeated experiments at thermoneutrality using UCP1-knockout (KO) mice, a model with no BAT effector function. In mice treated with FGF21-Fc, energy expenditure enhancement was fully preserved without a change in food intake. In contrast, hFGF21-infused mice manifested a blunted energy expenditure elevation but food intake decreased. Resultant weight loss and glycaemic/lipid benefits were similar to those in wild type animals. Collectively, these findings led to the proposal that WAT browning and UCP1 are not required for pharmacological effects of FGF21 treatment.

These intriguing studies interrogating the mechanisms of FGF21-mediated metabolic benefits raise many questions. First, if WAT browning and/or UCP1 are not involved in weight loss, what are the precise mechanisms of FGF21-induced benefits? Second, given the remarkable efficacy of FGF21 in ameliorating dysmetabolism in animals, why were effects of FGF21 analogue only modest in humans (Gaich et al., 2013)? Finally, in a broader context, if WAT browning is merely an epiphenomenon of FGF21 treatment, does this smother the excitement over WAT browning as an anti-obesity strategy?

Recent studies have unveiled a functional BAT/WAT axis in mice. BAT paucity induces compensatory WAT browning sufficient to maintain resistance to obesity (Schulz et al., 2013) and marked WAT browning emerges in BAT-ablated mice to restore FGF21-mediated glycaemic improvement (Emanuelli et al., 2014). Thus BAT-WAT crosstalk exerts determinative *in vivo* influence, indicating the need to consider BAT status when assessing the browning phenomenon. As iBAT was “whitened” in thermoneutral animals pre-treatment, FGF21 had induced browning of whitened

iBAT, despite relatively less browning of igWAT. Thermogenic effects of augmented iBAT could exceed igWAT browning because of greater UCP1 content (Nedergaard and Cannon, 2014). This implicates a browning hierarchy and browning of whitened iBAT may overshadow igWAT browning. In this regard, the observed similarity in metabolic benefits following FGF21 treatment at 21°C *vs*. thermoneutrality may be interpreted as congruent to "the sum of all browning" (*i.e*. browning of whitened iBAT + browning of igWAT), and total browning is required for maximum FGF21 pharmacological efficacy [Figure 1].

So why were FGF21-mediated benefits preserved in the absence of UCP1? FGF21-Fc recapitulated metabolic benefits primarily through energy expenditure enhancement. This represents UCP1-independent thermogenesis, ascribed to multiorgan mitochondrial PGC-1α enrichment (Veniant et al., 2015), as distinguished from UCP1-dependent pathways (*i.e*. WAT browning) in wild type animals. In contrast, UCP1-independent thermogenesis is blunted in mice treated with hFGF21 infusion, and decreased food intake contributes to total weight loss (Samms et al., 2015). These findings suggest the recruitment of UCP1-independent thermogenesis and appetite suppression may be FGF21 formulation dependent.

FGF21 is known to potentiate fasting and torpor centrally (Inagaki et al., 2007). Since hFGF21 can enter the central nervous system (CNS), centrally acting hFGF21 may quench appetite and thermogenesis. These anorexic and torpid signals may have been masked by FGF21-boosted UCP1-dependent thermogenesis in wild type animals. We do not know if FGF21-Fc crosses blood-brain-barrier. These responses may be lost if FGF21-Fc does not enter the CNS. The corollary of this hypothesis is whether FGF21

analogue design could be trading CNS penetrance for superior peripheral pharmacokinetics at the expense of attenuated appetite-suppressive benefits. Future studies of FGF21 analogues should address their central *vs*. peripheral actions on appetite *vs*. energy expenditure.

In a broader context, how do these findings shape the prospect of FGF21-based therapy in humans? Human BAT (hBAT) expresses UCP1, so humans are not UCP1-KO animals. The recruitability of UCP1-independent thermogenesis by FGF21 in humans is unclear. In contrast, hBAT possesses features of brite/beige fat (Jespersen et al., 2013), indicating modern humans are more closely resembling mice at thermoneutrality. hBAT is therefore more similar to brown-able "whitened iBAT" or igWAT. Indeed, WAT browning is inducible by FGF21 in human adipocytes (Lee et al., 2014) and cold acclimation recruits hBAT *in vivo* (Yoneshiro et al., 2013). Taken together, efficacy of FGF21-based therapeutics may ultimately hinge on browning in humans, despite their astonishing versatility in eliciting UCP1-independent thermogenesis. Since circulating FGF21 increases in humans following cold exposure, and FGF21 rise is greater among those with higher hBAT abundance (Lee et al., 2014), it is tempting to speculate hBAT abundance/responsiveness could modulate FGF21 effects. Recent clinical trial of FGF21 analogue treatment in humans did not show significant glycaemic benefits (Gaich et al., 2013). Could anti-diabetic effects be more prominent among selected individuals with greater hBAT abundance? It would be interesting to investigate whether hBAT status in humans impacts FGF21 analogue therapeutic efficacy in the future.

Overall, these new studies challenge the view that WAT browning underlies FGF21-mediated pharmacological effects. This dissociation may have been clouded by "hierarchical browning" of whitened BAT as the "sum of all browning" may be responsible, at least partly, for the efficacy of FGF21 therapeutics. With multiple FGF21 analogues poised for clinical trials in the era of hBAT Renaissance, it will be important to determine the contribution of hBAT activation and/or recruitment to the observed effects.

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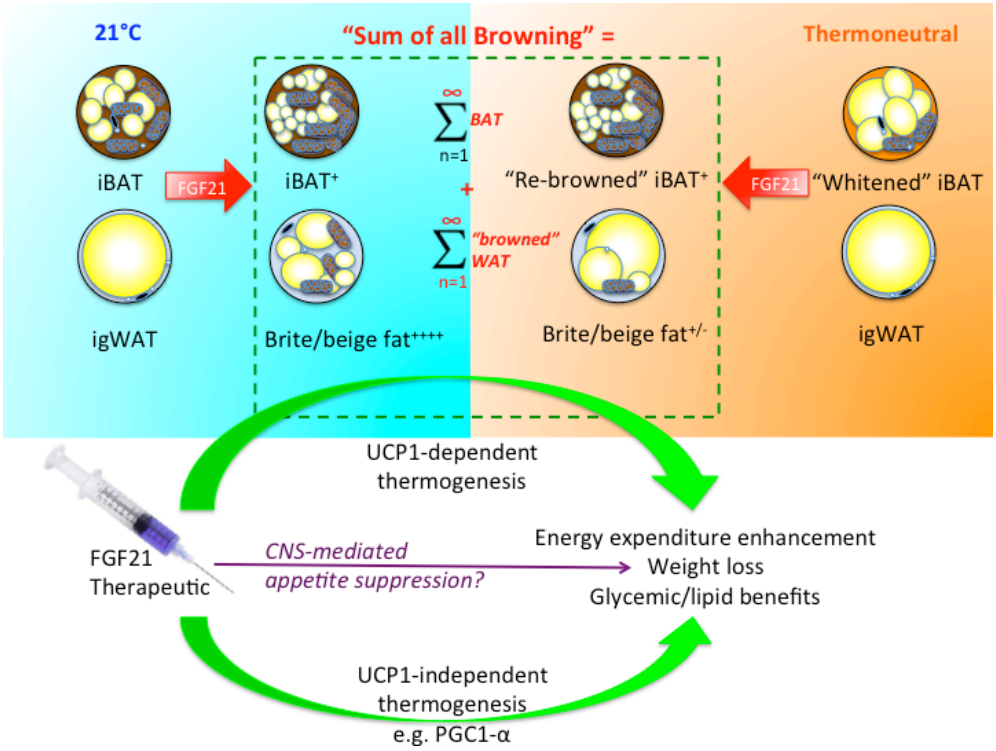
Figure legend

Figure 1. Schematic diagram illustrating interplay between central/peripheral FGF21 actions, thermogenesis, interscapular BAT (iBAT) and inguinal WAT (igWAT). The efficacy of FGF21 therapeutics is mediated via i) UCP1-dependent thermogenesis (i.e. igWAT browning + re-browning of whitened iBAT resulting in overall boosting of whole body BAT function), ii) UCP1-independent thermogenesis (e.g. stimulation of multi-organ mitochondrial biogenesis and PGC-1α enrichment) and iii) central nervous system (CNS) mechanisms.

Figure 1


21°C
"Sum of all Browning" =
Thermoneutral
iBAT
FGF21
iBAT+
∑ n=1 BAT
+
"Re-browned" iBAT+
FGF21
"Whitened" iBAT
igWAT
∑ n=1 "browned" WAT
Brite/beige fat++++
Brite/beige fat+/-
igWAT
UCP1-dependent thermogenesis
FGF21 Therapeutic
CNS-mediated appetite suppression?
Energy expenditure enhancement
Weight loss
Glycemic/lipid benefits
UCP1-independent thermogenesis e.g. PGC1-α