A comparative study of fatty acid amide hydrolase activity between three adipose depots in Zucker obese and Zucker diabetic rats

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Visceral and subcutaneous adipose tissues are metabolically distinct, but there are conflicting reports as to whether there is differential distribution of endocannabinoid system (ECS) components (1,2). Endocannabinoids in the circulation are upregulated in obesity, and gene transcription of ECS components in adipose has been reported to be both upregulated (2) and downregulated in obesity (3). Additionally, fatty acid amide hydrolase (FAAH, catalyses anandamide (AEA) hydrolysis) activity, as opposed to mRNA, has not been investigated in adipose tissue. Many obese subjects have metabolic disorders, but any relationship between diabetes and ECS activity in adipose tissue remains largely unreported. This study aims to determine whether FAAH activity differs between subcutaneous and visceral adipose depots in Zucker rat models of obesity and type 2 diabetes.

Male Zucker (*fa/fa*) and Zucker diabetic rats (299-384g, Charles River) were killed by cervical dislocation, and subcutaneous abdominal, epididymal and visceral adipose depots were dissected immediately. Tissues were digested with collagenase to release mature adipocytes, which were then homogenised and centrifuged (20,000 *g*, 20 minutes). The particulate and cytosolic fractions were stored at -80^{o}C until assay of FAAH activity using 2 µM *N*-arachidonoyl-^{3}H-ethanolamine as substrate (4). Data were analysed by one-way ANOVA and Bonferroni's *post hoc* test, and are reported as mean±S.E.M.

FAAH activity was detected in the particulate but not cytosolic fractions and was completely eradicated in the presence of URB597 (1 µM). No significant difference in FAAH activity was found between the three adipose depots in either group of rats, but Zucker diabetic rats showed significantly lower FAAH activity in the abdominal adipose depot than Zucker obese rats (17.4 ± 5.5 vs 50.8 ± 10.7 pmol/min/mg protein respectively, $P<0.05$; n=8). There was a trend for the diabetic rats to have lower FAAH activity in the epididymal depot than the obese rats (9.6 ± 2.6 vs 29.4 ± 5.1 pmol/min/mg protein), but this failed to reach significance.

Regression analysis revealed that in the Zucker diabetic rats there was a non-significant trend for weight to inversely correlate with FAAH activity in adipocytes from abdominal ($P=0.089$) and subcutaneous ($P=0.065$) depots. The inclusion of the Zucker obese rats in these analyses led to a positive correlation between weight and abdominal FAAH activity ($P=0.100$), indicating that FAAH activity varies between subgroups of obese animals.

The main finding of this study is that FAAH activity does not differ between adipose sites within the same animal. Also, FAAH activity is decreased in the abdominal visceral adipose tissue of obese diabetic rats compared to obese rats, and it is therefore possible that endocannabinoid signalling in adipose is affected by diabetes.

1. Blüher, M *et al*. (2006) Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 55:3053-3060.
2. Pagano, C *et al*. (2007) The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms. *J Clin Endocrinol Metab* 90:4810-4819.
3. Engeli, S *et al*. (2005) Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 54:2838-2843.
4. Boldrup, L *et al*. (2004) A simple stopped assay for fatty acid amide hydrolase avoiding the use of a chloroform extraction phase. *J Biochem Biophys Methods* 60:171-177.