SUPPLEMENTARY MATERIALS

A randomized cross-over trial assessing the effects of acute exercise on appetite, circulating ghrelin concentrations and butyrylcholinesterase activity in normal weight males with variants of the obesity-linked *FTO* rs9939609 polymorphism.

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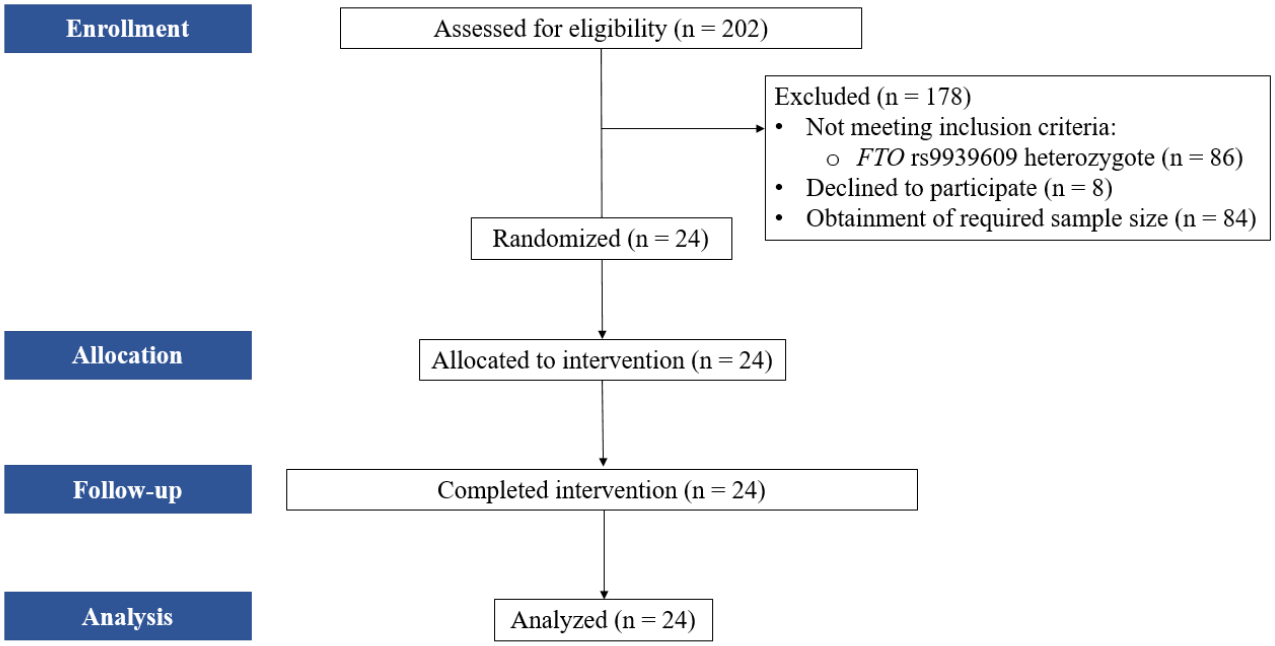
Supplementary Materials

Participant flow chart



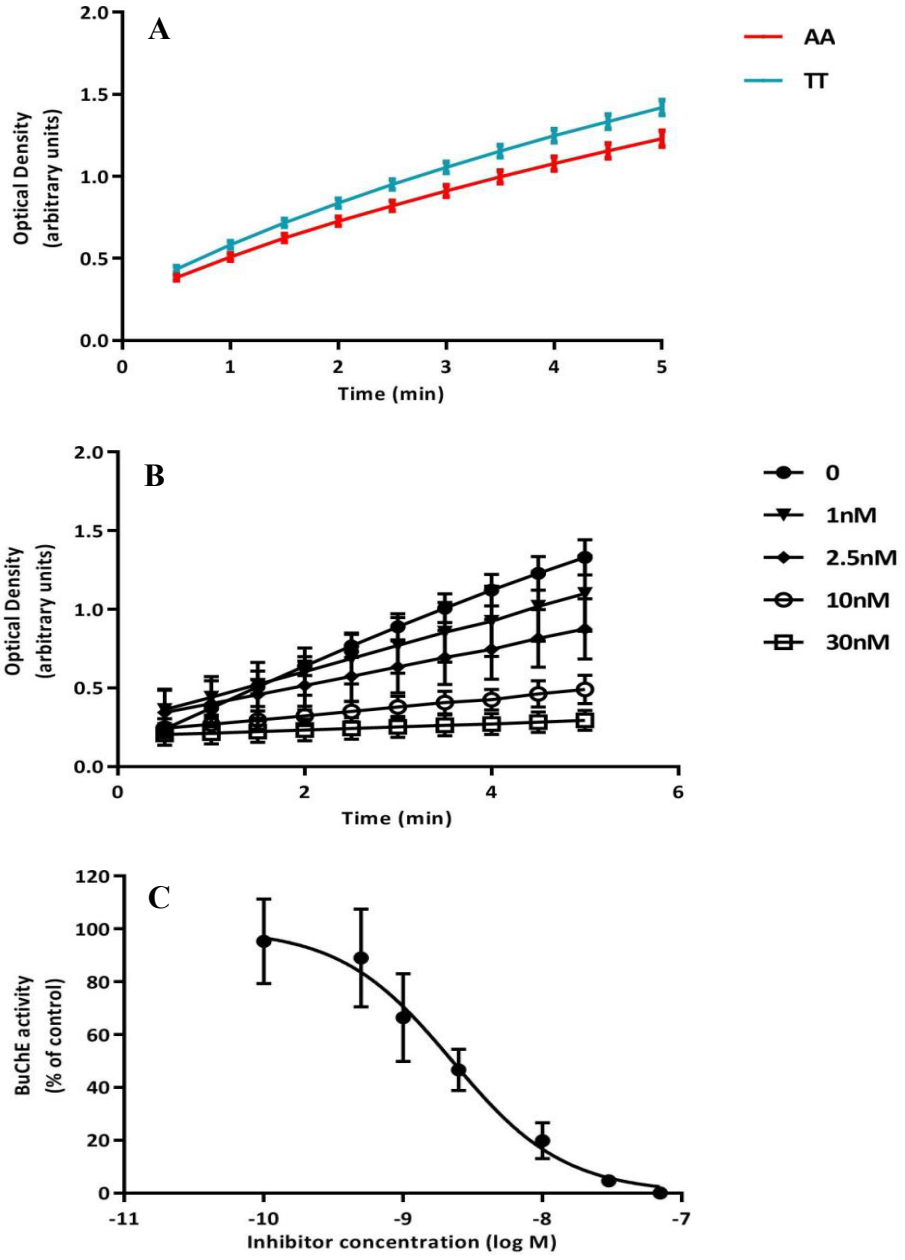
Supplementary Figure 1. Participant flow chart.

SUPPLEMENTARY METHODS

Butyrylcholinesterase activity

Butyrylcholinesterase (BChE) assays were performed based upon the cholinesterase assay method developed by Ellman (1), with butyrylthiocholine iodide as the enzymatic substrate. To quantify BChE activity, 3 µL of plasma was mixed with 150 µL 0.38 mM 5,5'-dithiobis-(2-nitrobenzoic acid), and 43 µL of 10 mM Tris-HCL (pH 8.0). Four µL of 35 mM BTCI was added to initiate the reaction. The production of the 5-thio-2-nitrobenzoate anion was followed at 410 nm, with readings taken every 30 s for 5 minutes using a Thermo Multiskan plate reader. All data points were performed in duplicates, from which an average value was determined. Blank readings due to either an absence of substrate or plasma were subtracted from final readings. The rate of production of the 5-thio-2-nitrobenzoate anion was linear over the 5 minute assay with typical R^2 values of 0.98-0.99 (Supplementary Figure 2A).

To measure BChE inhibition, assays were performed as above except 10 µl of ethopropazine hydrochloride in dimethylsulfoxide, or vehicle, replaced 10 µl of the Tris-HCl buffer. A dose response of BChE inhibition by ethopropazine hydrochloride was performed over the concentration range of 0.1-100 nM (Supplementary Figure 2B), with the concentration of ethopropazine producing 50% enzymatic inhibition (IC_{50}) calculated as 2.3 nM using non-linear regression with GraphPad Prism 7.03 (Supplementary Figure 2C). For calculation of BChE activity, a molar absorption coefficient of 14140 M/cm was used and a light pathlength of 0.56 cm (2).



Supplementary Figure 2. Averaged rate of production of the TNB anion in AAs and TTs during the 5 minute assay (A), Dose response of BChE inhibition by ethopropazine hydrochloride (B) and Dose response of BChE inhibition by ethopropazine hydrochloride calculated using nonlinear regression, with BChE activity in the absence of inhibitor set at 1 (C). Data are represented as mean ± SEM.

References

1. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.

2. Eyer P, Worek F, Kiderlen D, Sinko G, Stuglin A, Simeon-Rudolf V, Reiner E. Molar absorption coefficients for the reduced ellman reagent: Reassessment. Anal Biochem. 2003;312:224–7.