Exploring Autoimmune Features in Type 2 Diabetes: A study on Autoantibody Prevalence Against GLP-1

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Introduction

Type 2 diabetes mellitus (T2DM) is characterised by the presence of insulin resistance and impaired glucose regulation. GLP-1 (glucagon-like peptide-1) is an incretin hormone which, alongside the other incretins, is responsible for around 70% of total insulin secretion in the body (reference). This poses a possibility of abnormal GLP-1 levels and/or the presence of autoantibodies against this hormone being a contributing factor to the symptoms seen in such conditions.

Currently limited study has been carried out regarding this and other GLP-1 differences between T2DM and healthy populations, thus furthering increasing demand around information about this unknown relationship and involvement.

The aim of this study was to compare the serum GLP-1 autoantibody levels between diagnosed T2DM individuals and a similarly matched healthy control group.

Abstract

A laboratory-based case-control study using blood samples from a total of 85 individuals of either type 2 diabetic diagnosis or of healthy status, broadly selected for similar distribution in age and sex. Antibody levels were quantified through an enzyme-linked immunosorbent assay (ELISA).

Data obtained was then further analysed through usage of Prism and R, with propensity matching being carried out to account for comorbidities such as arthritis, cancer and hypertension.

Mean GLP-1 absorbance values showed a slight difference between the type 2 diabetes population and the controls (mean 2.07 vs 2.74, respectively). However, this was not found to be significant (p = 0.25, Welch's t test). The study used sample sizes of just over 40 per group (n = 85) and thus through power analysis it was indicated that the study was only powered to detect medium-to-large effect sizes. As the observed effect size was small (η2 = 0.019), this suggests that the study was underpowered to detect subtle differences.

At the present sample size, GLP-1 antibody levels can not be concluded to differ significantly between type 2 diabetes and control cohorts. The small effect size and limited power highlight the requirement of further larger-scale investigations to better define the role of GLP-1 and its immunological involvement in type 2 diabetes.

Materials and Methods

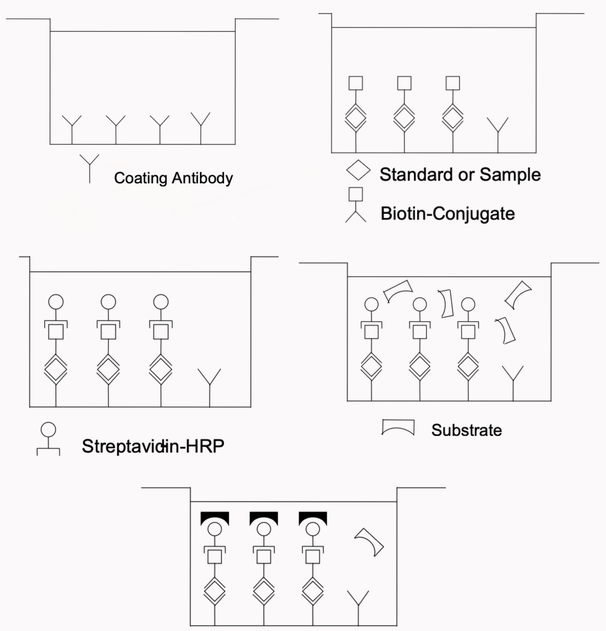


Figure adapted from ThermoFisher Human GLP-1 ELISA Kit Manual, category #BMS2194

The study design followed a case-control methodology involving the bloods of 45 T2DM diagnosed and 40 healthy participants (n = 85).

Participants were selected to be over the age of 50 and were further matched broadly regarding characteristics such as age and sex. Propensity matching was carried out using R (version 4.5.1) to ensure effective matching and validity in group comparisons. These blood samples were stored at 80°C until sub-aliquoting to allow for appropriate and efficient use.

The assay was carried out in single using a commercial ThermoFisher sandwich ELISA kit for measuring antibodies present against GLP-1. The absorbencies obtained from this kit after following were measured at a wavelength of 450 nm.

A standard curve was generated from known GLP-1 concentrations developed on the same kit.

To allow for analysis, concentrations were interpolated from the curve and statistical comparison through usage of a t-test on the software Graphpad Prism (version 10) was carried out. The significance was set at $p < 0.05$.

Results

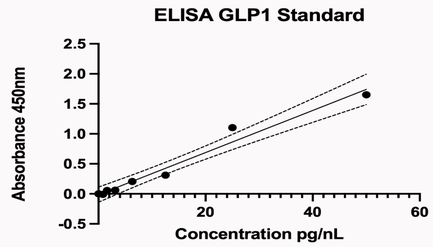
Findings of this assay were found to be that mean GLP-1 absorbance in the T2DM population measured were 2.07, and that of the control population was 2.74. This resulted in a mean difference of 0.67 (± 0.56). This was found to be non-significant a p value of 0.25 and a 95% confidence interval of -0.47 to 1.80.

Variance homogeneity was further assessed using an F-test however variability did not show to differ significantly between the two groups (F (39,39) = 1.83, p = 0.064). The calculated effect size was also found to be small with it being discovered to be $\eta^2 = 0.019$.

Power analysis curves were also generated alongside results to assess the sensitivity of the study, and it was found that with groups of sample size 40 and 45, the study was sufficiently powered at 80% to detect effects of moderate-to-large sizes: Cohen's d ≥ 0.6 – 0.7.

These results show a slight trend towards a lower GLP-1 in T2DM, but this was not found to be statistically different within a sample size of n = 85.

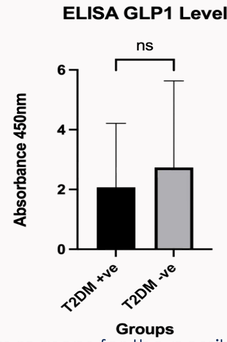
Test	Value	Result
Mean absorbance (T2DM)	2.07	
Mean absorbance (Control)	2.74	
Mean difference ± SEM	0.67 ± 0.56	
95% CI	−0.47 to 1.80	Not significant
Welch's *t* (df=71)	1.17	*p* = 0.25
F-test for variances	F(39,39) = 1.83	*p* = 0.064
Effect size (η^2)	0.019	Small



Discussion

It can be concluded from the results obtained that for the power available with the sample size (n = 85), no significant difference was observed between the two groups.

Serum GLP-1 was detectable in both groups and mean absorbance was lower in T2DM patients compared with controls, but this was not statistically significant (p = 0.246, $R^2 = 0.019$). These results show that there is a possibility of a a higher GLP-1 antibody presence in control groups in comparison to T2DM, however that variability is high in these groups. The small effect size however indicates that the group status likely explains little of the variance.



There are various reasons for the possible cause of this result. The most likely reason is the underpowered nature of the assay being unable to detect subtle differences. It is possible there is in fact a difference between these groups however greater sensitivity may be required to record this. In order to compensate for this a larger sample size should be used in future assays carried out researching this. Another hypothesis explaining the minor difference seen is the possibility of biological variability. Limited information regarding the diet, medication and other lifestyle factors which may result in altered results. Further, as with all ELISAs, the possibility of cross-reactivity with similar peptides may have reduced accuracy in measurements obtained.

Conclusion

GLP-1 was measurable in both T2DM and control groups, however no significant difference was found between the groups in this cohort. Findings point towards the possible presence of a subtle GLP-1 differences however larger studies are required for confirmation of this.

Future work in this field should involve larger, multi-centre cohorts. Ideally, measurement of GLP-1 would also involve both analysis into active and total GLP-1 in the body as well as functional incretin response assays; thus, allowing for better comprehension to the role difference if present between these two groups.

Acknowledgments

I would like to sincerely thank my supervisor Dr Eoin McKiney, the support of Daniel Griffiths, and finally the Laidlaw foundation for allowing me to carry out such research and gain much valuable insight in this domain.