Table 59: Baseline characteristics of randomised participants. Data are means ± SE unless stated otherwise. P value for melatonin subjects versus control subjects (independent two-samples t-test) is considered significant if it is below 0.05. In bold are parameters that were significantly different (red blood cell distribution width (RDW)) or of borderline significance (HbA1c and mean corpuscular haemoglobin (MCH)) between the two intervention arms at baseline

Parameter	Melatonin	Placebo	p
Female, *n (%)*	31 (83.78)	28 (73.68)	0.29
Age (years)	47.08 (15.63)	47.08 (12.51)	1.00
Height (m)	1.67 (0.07)	1.67 (0.09)	0.92
Weight (kg)	76.34 (14.39)	77.75 (15.27)	0.68
BMI	27.31 (4.95)	28.10 (4.79)	0.49
Waist (cm)	88.38 (13.70)	90.32 (13.14)	0.54
Hips (cm)	107.00 (10.39)	106.56 (9.47)	0.85
BP systolic (mm/Hg)	121.49 (14.26)	123.26 (18.27)	0.64
BP diastolic (mm/Hg)	81.46 (10.39)	80.26 (12.51)	0.65
HR (bpm)	72.78 (13.04)	70.68 (9.24)	0.43
HbA1c (mmol/mol)	34.97 (4.69)	37.09 (4.08)	**0.05**
Tot Chol (mmol/L)	5.12 (0.86)	5.03 (0.91)	0.66
Ratio chol:HDL	3.45 (1.14)	3.36 (0.83)	0.70
HDL(mmol/L)	1.63 (0.42)	1.55 (0.36)	0.41
LDL(mmol/L)	3.04 (0.79)	2.96 (0.82)	0.68
Trig (mmol/L)	1.08 (0.68)	1.16 (0.76)	0.66
CRP (mg/L)	2.39 (7.10)	2.90 (5.40)	0.74
Gluc (mmol/L)	4.75 (0.47)	4.95 (0.58)	0.10
Sodium (mmol/L)	140.39 (1.85)	141.22 (2.87)	0.18
Potasium (mmol/L)	4.30 (0.26)	4.39 (0.27)	0.15
Creatinine (umol/L)	64.33 (10.66)	69.24 (10.99)	0.06
eGFR (mL/min/1.73m^2)	78.00 (8.02)	78.74 (8.29)	0.80
Urea (mmol/L)	5.05 (1.23)	5.55 (1.31)	0.10
Bilirubin (umol/L)	11.92 (5.79)	11.15 (5.49)	0.57
ALT (iu/L)	22.56 (7.41)	28.94 (25.06)	0.16
Albumin (g/L)	44.72 (2.85)	43.82 (2.54)	0.17
Calcium-adjusted (mmol/L)	2.31 (0.08)	2.30 (0.06)	0.39
AST (iu/L)	23.28 (4.28)	26.21 (14.17)	0.26
Gamma GT (iu/L)	24.72 (17.41)	29.71 (12.12)	0.43
Neutrophil (10*9/L)	3.59 (1.16)	3.54 (1.59)	0.87
Lymphocyte (10*9/L)	1.75 (0.55)	1.83 (0.67)	0.58
Monocyte (10*9/L)	0.35 (0.15)	0.35 (0.13)	0.97
Eosinophil (10*9/L)	0.17 (0.11)	0.15 (0.08)	0.33
Basophil (10*9/L)	0.05 (0.04)	0.05 (0.04)	0.87
Haemoglobin (g/L)	139.22 (10.77)	139.26 (15.85)	0.99
WBC (10*9/L)	6.05 (1.43)	6.02 (2.05)	0.93
Platelets (10*9/L)	261.39 (54.23)	247.11 (62.50)	0.31
MCV (fl)	93.47 (5.07)	91.31 (6.59)	0.13
PCV	0.42 (0.03)	0.43 (0.04)	0.52
RBC (10*12/L)	4.55 (0.36)	4.73 (0.53)	0.10
MCH (pg)	30.66 (2.01)	29.55 (2.52)	**0.05**
RDW	12.96 (0.94)	13.57 (1.12)	**0.02**
vWF (ng/ml)	12010.25 (3453.26)	12102.11 (5190.33)	0.93
FXII (ng/ml)	23004.56 (2429.04)	23539.09 (2958.59)	0.41
PF4 (ng/ml)	448.50 (193.81)	487.94 (133.76)	0.32

Task 3.3 – Bioinformatics analysis, pathway mapping, and data mining analysis of complete data sets on lifestyle interventions in first degree relatives

Out of 75 participants randomised, 71 patients, 64 patients and 60 attended visit 1, 2 and 3, respectively (Figure 45). A total of 60 patients completed the final follow-up visit at 9 months.

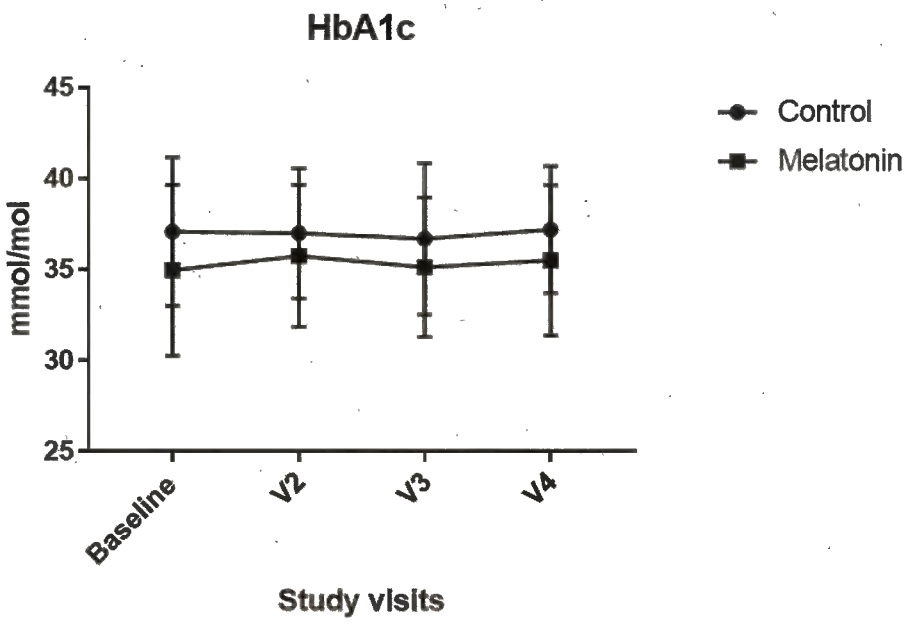
Baseline characteristics

The baseline characteristics of the study population are presented in Table 59. The mean age was 47.08 ±14.04 years and 21% were males. Approximately 69% were either overweight or obese (BMI≥25.0). There was no significant difference in gender, age, BMI, blood pressure and glucose level between the two treatment groups at baseline, ($p > 0.05$). Furthermore, baseline HDL, LDL and triglycerides concentrations were similar between the two groups ($p > 0.05$).

Mean level of HbA1c was higher in the placebo group compared to the melatonin group, but the difference failed to reach statistical significance (p=0.05).

Primary Outcomes

There were no differences in HbA1c in individuals treated with melatonin compared to placebo and similarly no differences in glucose tolerance at any time point during the OGTT (Figure 46).



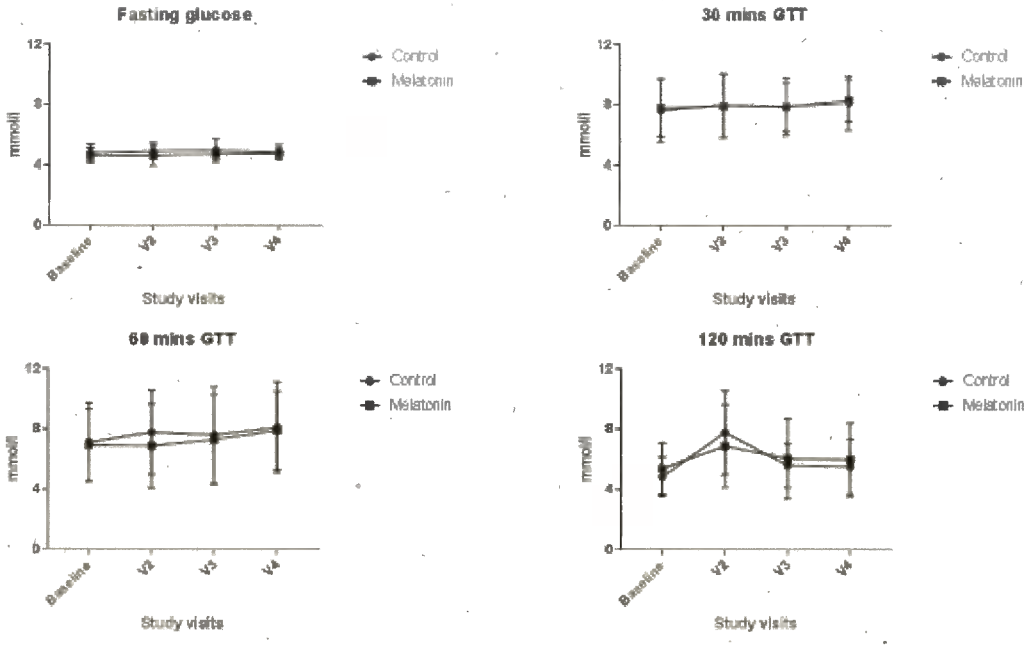


Figure 46: Primary outcome of the study: HbA1c and serum glucose concentration (fasting and during an oral glucose tolerance test performed at each of the 4 study visits

Secondary Outcomes

i) Weight and blood pressure

There were no differences in weight or blood pressure at any time point in the melatonin arm compared to placebo (Figure 47).

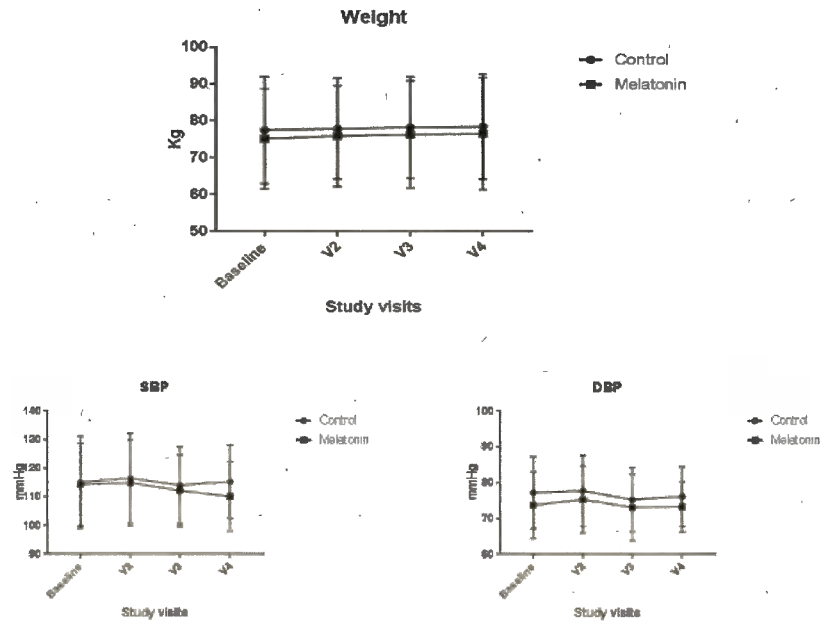
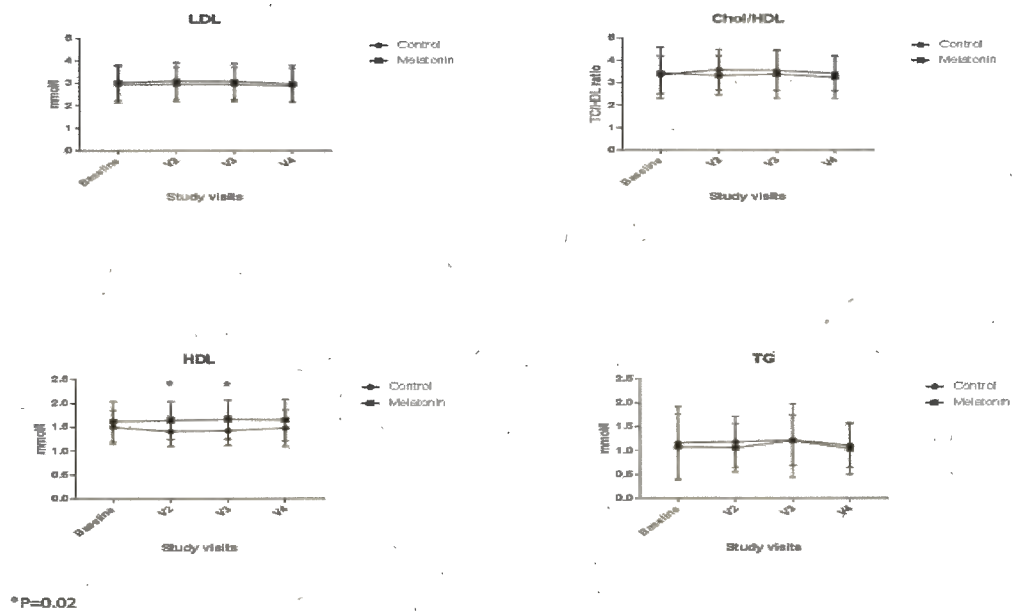


Figure 47: Body weight, systolic blood pressure and diastolic blood pressure in the melatonin and placebo groups

ii) Lipids

There were no changes in total cholesterol or LDL cholesterol between the treatment and placebo arms. There was a borderline statistically significant increase in HDL cholesterol in the melatonin treated group, but this appears to be due to a fall in the placebo group rather than an increase in the melatonin arm (Figure 48).



*P=0.02

Figure 48: Lipid profiles in the melatonin and placebo groups

iii) Inflammation and thrombosis

There were no significant changes in any markers of inflammation or thrombosis including PAI-1, Factor VII, D-Dimer and thrombin anti thrombin complexes (data not shown). There were no differences in fibrinogen, CRP, or measures of fibrin clot structure (Figure 49).

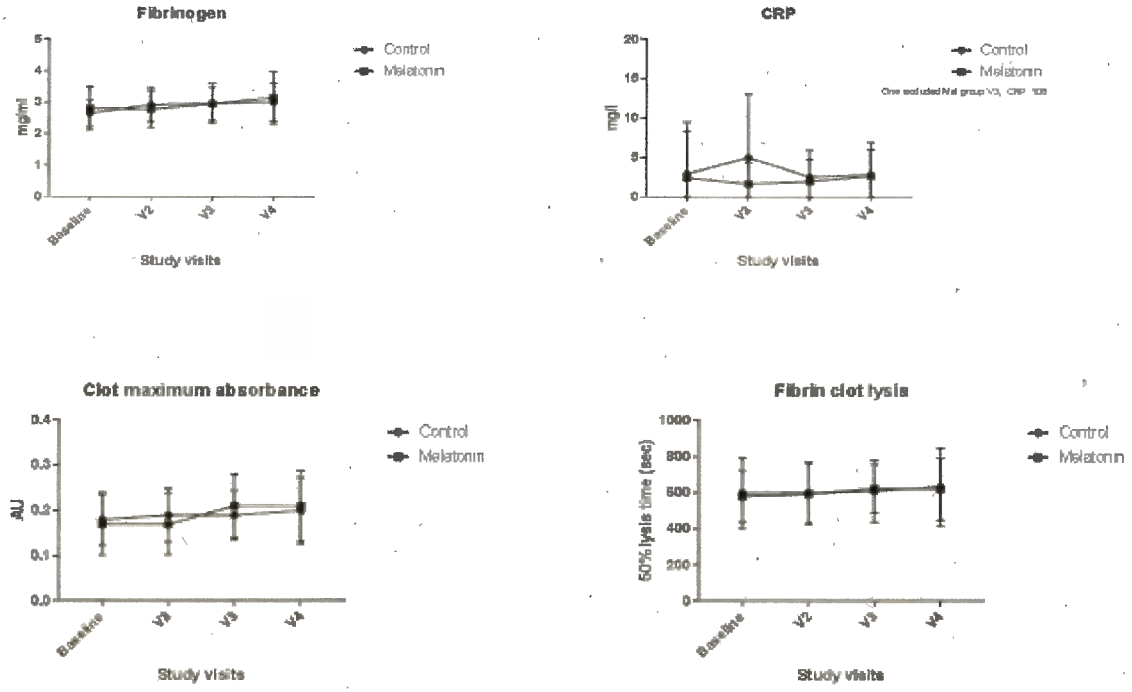


Figure 49: Body weight, systolic blood pressure and diastolic blood pressure in the melatonin and placebo groups

Effects of melatonin therapy on vWF, FXII and PF4

Although a reduction in in vWF was apparent after melatonin therapy, the difference compared with the placebo group was not significant. (p=0.82). Three months after treatment was commenced, both the FXII and PF4 levels concentrations in the melatonin group were lower than in the placebo group (p=0.05). These differences were not sustained after treatment was discontinued.

Adjusted treatment effect on vWF, FXII and PF4

Pairwise correlation analysis was performed to inform multiple linear regression model used to assess the unbiased treatment effect of melatonin at the end of the treatment and 3 months post-treatment. An arbitrary cut-off point of 0.7 for Pearson correlation coefficient was chosen. There were strong positive correlations between a number of parameters, including weight (kg), BMI, waist (cm) and hips (cm), glucose (mmol/L) and HbA1c, LDL (mmol/L) and total cholesterol (mmol/L), and between Hb (gl), RBC (10*12/L) count and PCV ($p<0.05$).

Taking into account the significant correlations identified, sex, age (years), BMI, systolic blood pressure (mmHg), HDL (mmol/l), LDL (mmol/l), CRP (mg/L), eGFR (mL/min/1.73m^2), ALT (iu/L), calcium-adjusted (mmol/L), WBC (10*9/L) and platelets (10*9/L) were chosen as the most important confounders and were used as independent variables in the multiple linear regression model. In addition, the model was adjusted for those variables that were of borderline significance/significantly different between the treatment arms at baseline (RDW, MCH and HbA1c).

Melatonin reduced levels of vWF, FXII and PF4 compared to placebo, with more pronounced reductions in the concentrations of the three markers in relation to baseline values observed at the end of the treatment period than at visit 4 (Figures 50-52). The average changes in vWF, FXII and PF4 concentrations for the melatonin group vs placebo between Visit3 or Visit4 and baseline values showed no statistically significant differences, i.e. the observed treatment effects of melatonin on the three of the markers measured (vWF, FXII and PF4) were non-significant, (p>0.05).

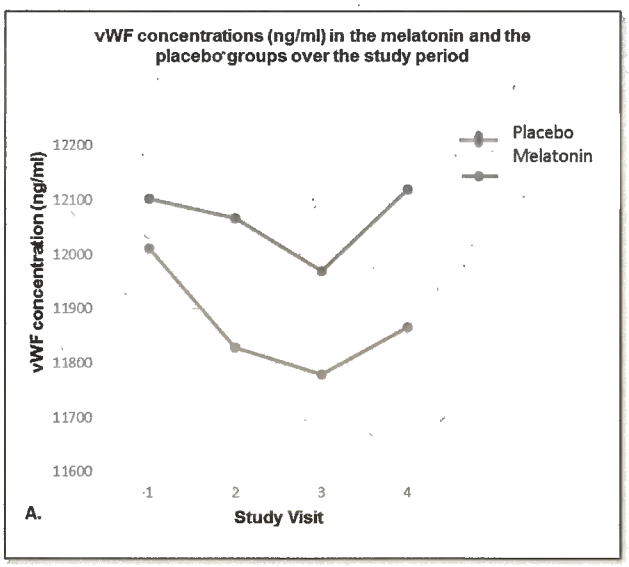


Figure 50: von Willebrandt factor concentration in the melatonin and placebo groups

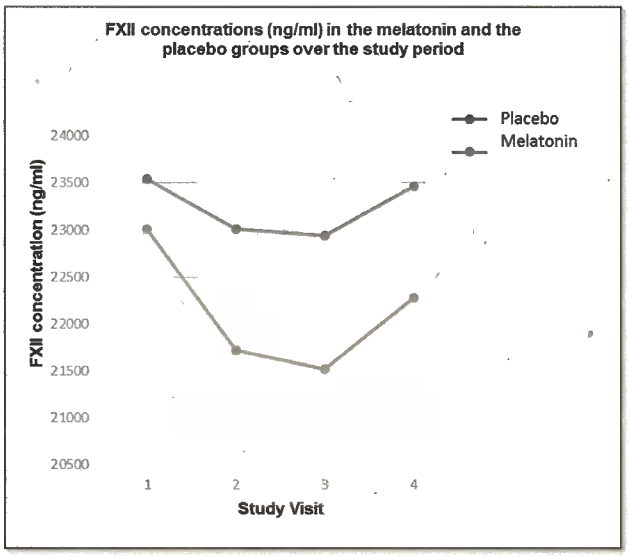


Figure 51: Factor XII concentration in the melatonin and placebo groups

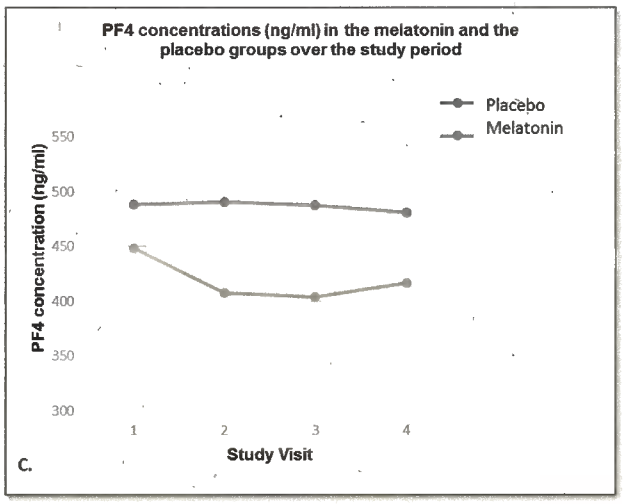


Figure 52: Platelet factor 4 concentration in the melatonin and placebo groups

IV) Clock gene expression

Clock genes were measured in the white cell fraction of whole blood by rtPCR. No differences in expression of any of the genes measured were observed with melatonin treatment (Figure 53).

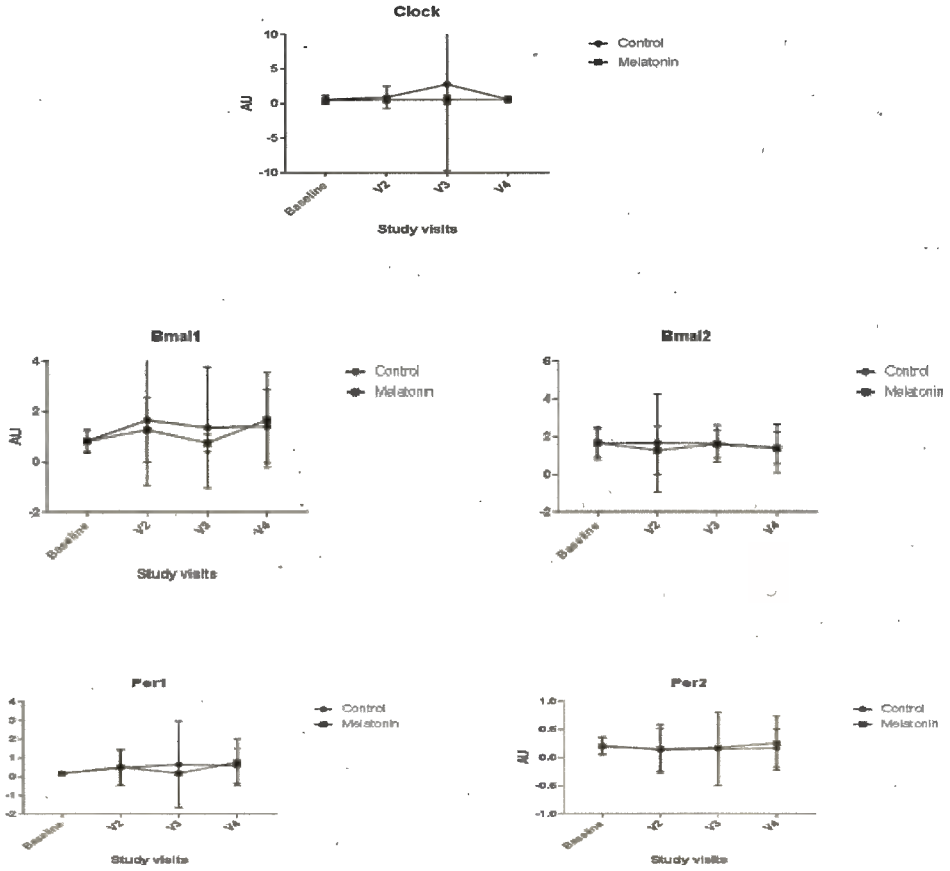


Figure 53: mRNA expression of the circadian clock genes Clock, Bmal1, Bmal2, Per1, and Per2 in the melatonin and placebo groups

Conclusions

The addition of melatonin 2mg nocte in a double blind placebo controlled trial in normoglycaemic first degree relatives of type 2 diabetes subjects had no outstanding effects on metabolic control of glucose.

In addition, there was a significant increase in HDL cholesterol at the two treatment points in the melatonin group ($p<0.05$) but this appeared to be due to a fall in the placebo group rather than an increase in the melatonin-treated arm. Results of thrombotic parameters were largely non-significant although there was a borderline significant reduction in the platelet marker PF4 and in levels of coagulation factor XII.

Statement on the use of resources

The major effort during this 4th reporting period was put into the successful completion of the intervention study in first degree relatives of persons with type 2 diabetes. This work was performed by investigators from Leeds, with strong support by co-investigators from Surrey and Genedata.