Work package 3 – Controlled, randomized chronotherapeutic lifestyle intervention studies in first degree relatives

As outlined in the previous report, recruitment for this study commenced in October 2014. Since that time we have had one nurse employed by the EU grant and one nurse employed on local funding working on recruitment. We received ethical approval to recruit subjects from General Practice clinics and we visited local temples to recruit ethnic minority subjects. Unfortunately there was a delay in receiving melatonin (as described in the previous report).

Task 3.1 – Recruitment of first degree relatives at baseline and intense phenotyping (planned: M06-M30)

This task has been completed, as described in the 3rd interim report.

Task 3.2 –Randomisation to light therapy plus melatonin, or light therapy or melatonin alone, or placebo alone (4 groups of 120 subjects each, 100 subjects to finalize each group)

According to changes described in the DoW approved in amendment 2 and outlined in our 2nd interim report, randomization in this work package is performed between melatonin treatment and placebo.

A total of 4000 patients were screened and 340 relatives of patients could be contacted. A total of 75 patients willing to participate were subsequently randomised in a 1:1 random allocation held by the Leeds Teaching Hospitals Trust pharmacy to either 6 months melatonin therapy (2mg prolonged-release melatonin tablet Circadin®) (n=37) or placebo (n=38). Circadin® or placebo were taken once daily in the evening 2h before sleep. The study team and patients were blinded to the randomization. The inclusion and exclusion criteria are given in table 58.

Table 58: Inclusion and exclusion criteria for the study

Inclusion Criteria	Exclusion Criteria
• Ability to give an informed consent to be a study participant • Age 18-75 • Males, females of non-child bearing potential (post-menopausal or 6 weeks post-sterilisation), females of child-bearing potential (on hormonal contraceptives/intrauterine devices/double barrier methods) • One or more first degree relative with an established diagnosis of T2DM • Absence of clinical symptoms and signs of infection • Absence of systemic disease (including T2DM, renal and liver impairment) • Stable doses of any type of medication (prescription or non-prescription) for 3 months prior to study • Not taking medication contra-indicated for use in conjunction with melatonin	• Pregnancy/breastfeeding • Females of reproductive age not using acceptable contraception method • Current illness that could prevent the participant from completing the study or serious chronic illness (heart failure, autoimmune diseases, cancer) • Renal insufficiency (eGFR < 60 ml/min) • Liver impairment • Intolerance or hypersensitivity to melatonin or related compounds • Use of melatonin, fluvoxamine, cimetidine, quinolones, carbamazepine, rifampicine, 5-methoxypsoralene or 8-methoxypsoralene within 4 weeks prior to inclusion in the study

Fasting blood samples were collected at 4 study visits, with the first taking place at randomisation (baseline or visit 1). Visit 2 and 3 were scheduled 3 and 6 months after randomisation respectively. All participants finished randomised treatment at visit 3. The final set of blood samples was taken at visit 4 (Figure 45).

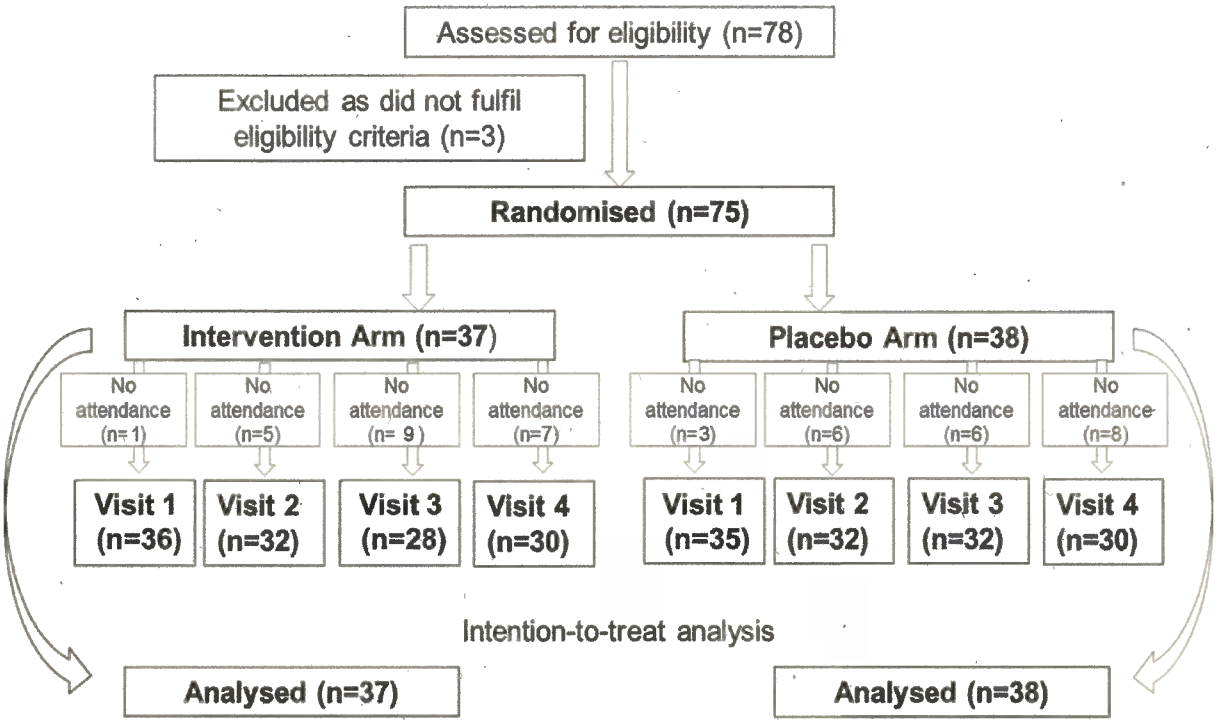


Figure 45: Flowchart for subject participation. 78 patients were screened and 75 patients were randomised (3.85% drop out) to either melatonin (n=37)) or placebo (n=38). Blood samples were taken from 36 patients randomised to melatonin and 35 patients randomised to placebo at Visit1. 32 patients receiving melatonin and 32 patients receiving placebo attended Visit 2 (3 months after randomisation). 28 patients receiving melatonin and 32 patients receiving placebo attended Visit 3 (6 months after randomisation). 30 patients receiving melatonin and 30 patients receiving placebo attended Visit 4 (9 months after randomisation. All patients stopped treatment 6 months after randomisation. The data were analysed according to the intention-to-treat concept based on the numbers randomised

Individuals were included in the study if they had at least one first degree relative with an established diagnosis of T2DM and were 18-75 years old. The participants were healthy subjects with no clinical symptoms or signs of infection or systemic disease, including T2DM, renal and liver impairment. Pregnant/breastfeeding females were excluded from the study (Table 59).

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.

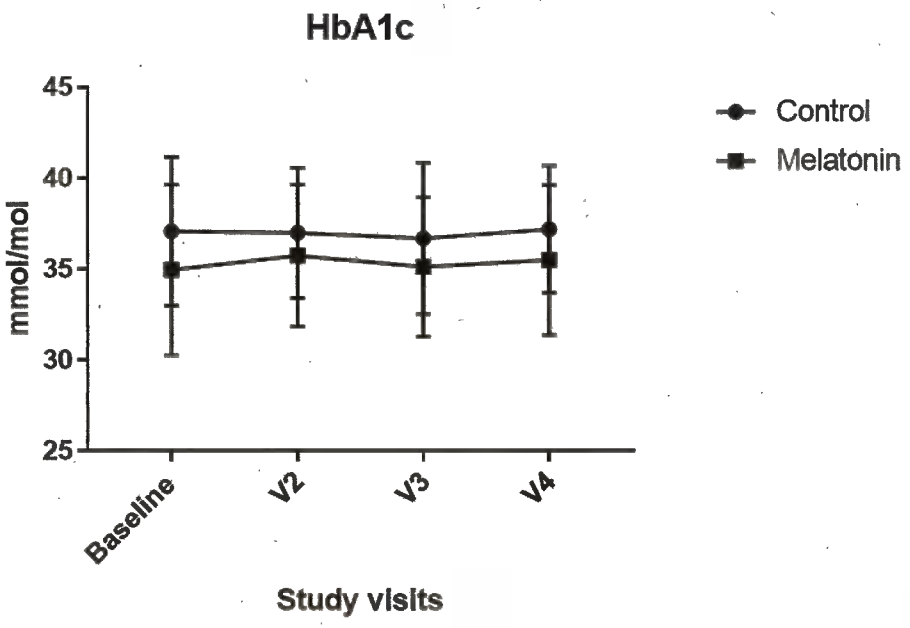
Baseline characteristics

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).



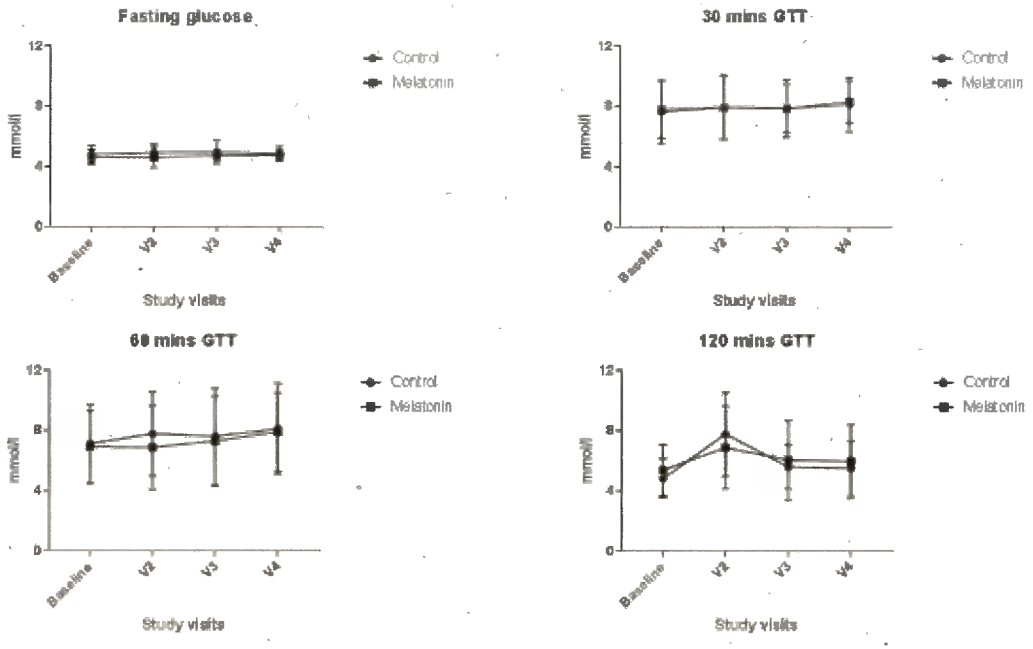


Figure 46: Primary outcome of the study: HbA1c and serum glucose concentration (fasting and during an oral glucose tolerance test performed at each of the 4 study visits

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).

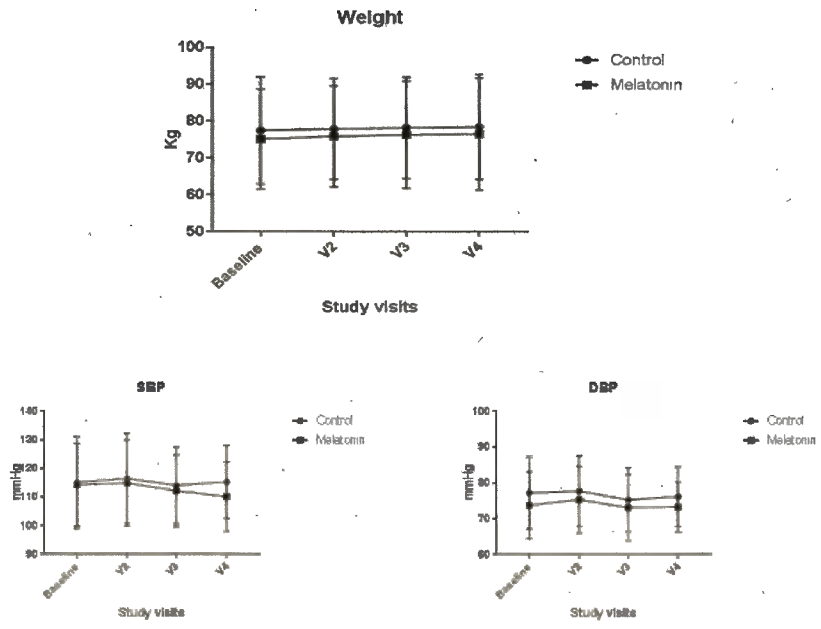


Figure 47: Body weight, systolic blood pressure and diastolic blood pressure in the melatonin and placebo groups

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).

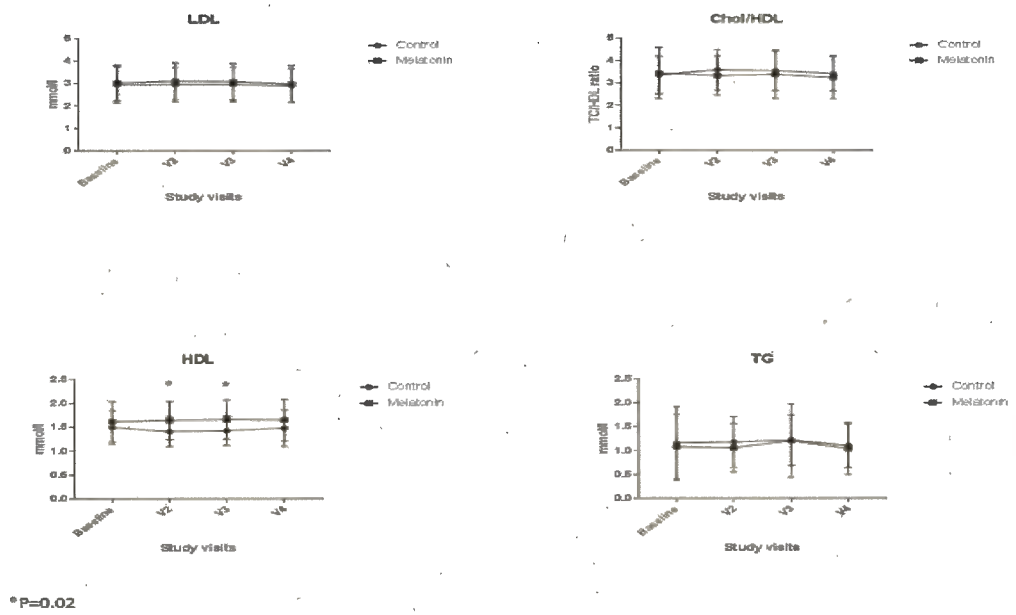


Figure 48: Lipid profiles in the melatonin and placebo groups

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).

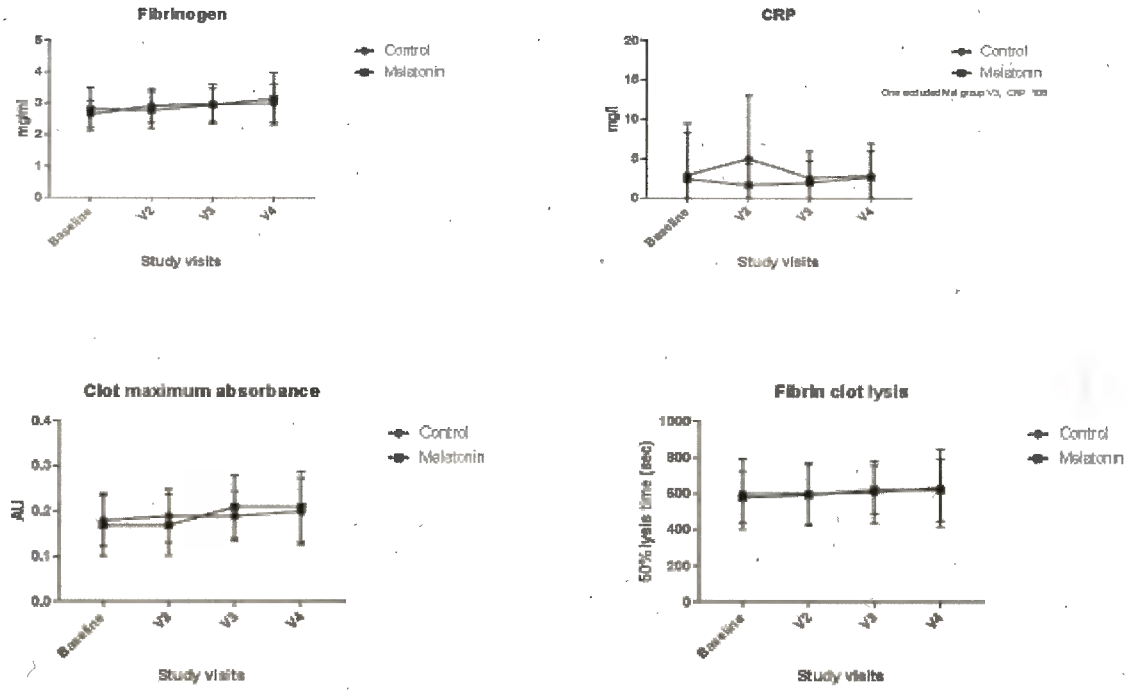


Figure 49: Body weight, systolic blood pressure and diastolic blood pressure in the melatonin and placebo groups

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).

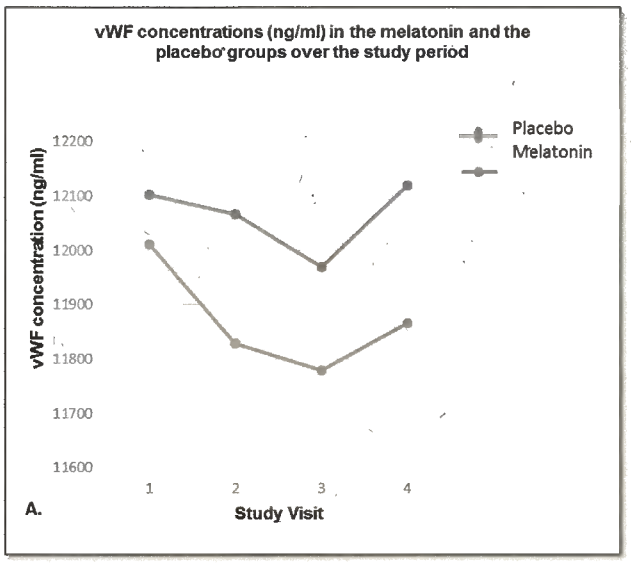


Figure 50: von Willebrandt factor concentration in the melatonin and placebo groups

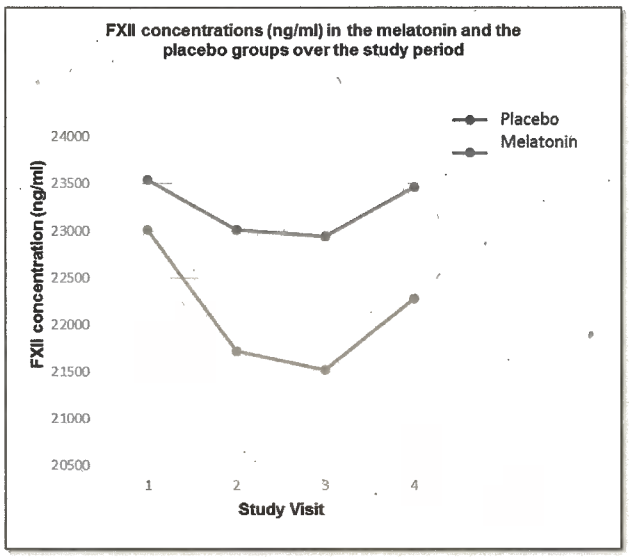


Figure 51: Factor XII concentration in the melatonin and placebo groups

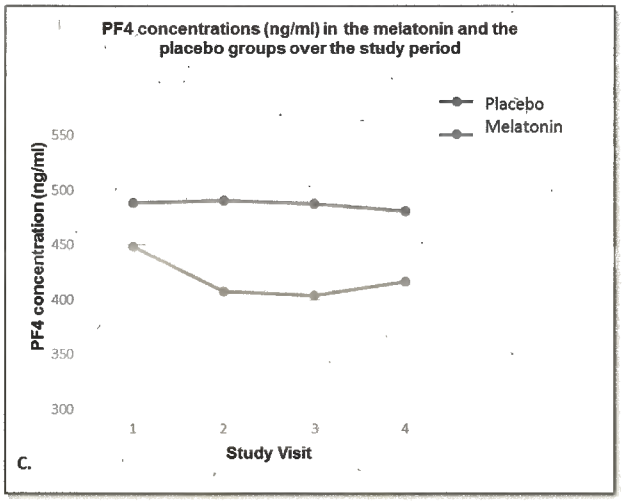


Figure 52: Platelet factor 4 concentration in the melatonin and placebo groups

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).

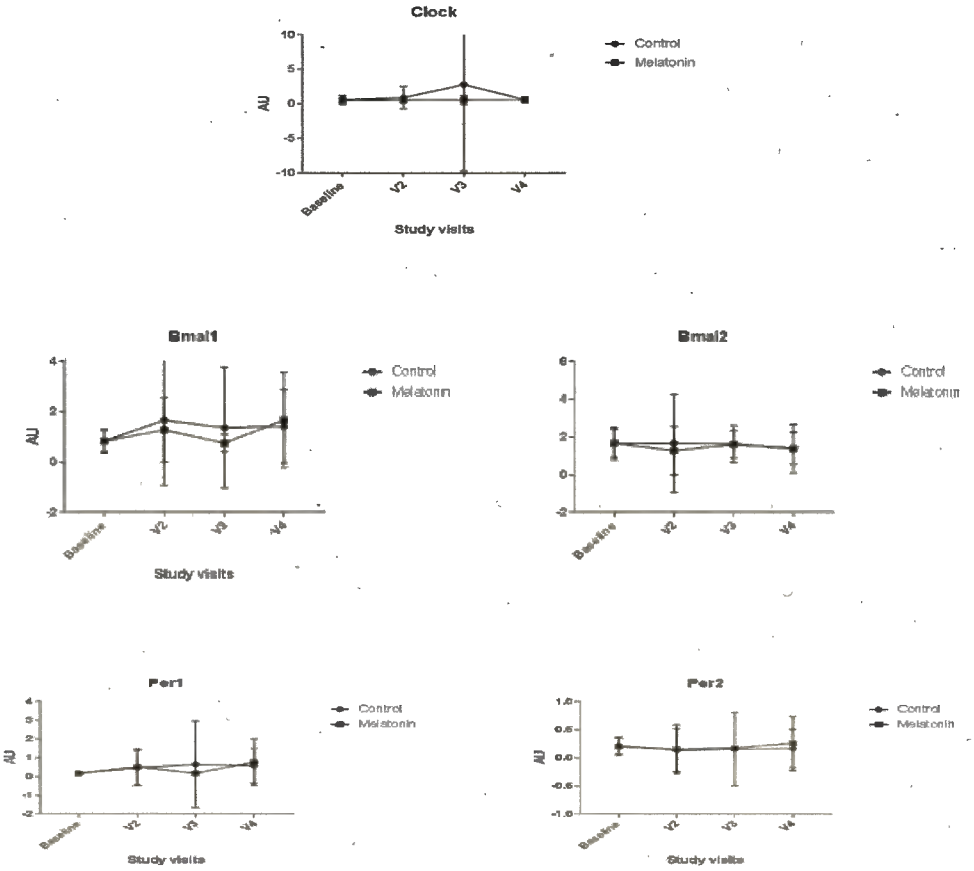


Figure 53: mRNA expression of the circadian clock genes Clock, Bmal1, Bmal2, Per1, and Per2 in the melatonin and placebo groups

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.