Glucose metabolism in healthy ageing

Wijsman, C.A.

CHAPTER 5

Ambulant 24-hour glucose rhythms mark calendar and biological age in apparently healthy individuals

Wijsman CA, van Heemst D, Hoogeveen ES, Slagboom PE, Maier AB, de Craen AJM, van der Ouderaa F, Pijl H, Westendorp RGJ, Mooijaart SP.

Abstract

Background. Glucose metabolism marks health and disease and is causally inferred in the aging process. Ambulant continuous glucose monitoring provides 24-hour glucose rhythms under daily-life conditions. We aimed to describe ambulant 24-hour glucose rhythms measured under daily-life condition in relation to calendar and biological age in apparently healthy individuals.

Methods. In the general population and families with propensity for longevity, we studied parameters from 24-hour glucose rhythms; glucose levels and its variability, obtained by continuous glucose monitoring. Participants were 21 young (aged 22 to 37 years), 37 middle-aged (aged 44 to 72 years) individuals from the general population, and 26 middle-aged (aged 52 to 74 years) individuals with propensity for longevity. All were free of diabetes.

Results. Compared to young individuals, middle-aged individuals from the general population had higher mean glucose levels (5.3 vs 4.7 mmol/L, p <0.001), both diurnally (p <0.001) and nocturnally (p = 0.002). Glucose variability was higher in the middle-aged compared to the young (standard deviation 0.70 vs 0.57 mmol/L, p = 0.025). Compared to middle-aged individuals from the general population, middle-aged individuals with propensity for longevity had lower overall mean glucose levels (5.2 vs 5.4 mmol/L, p = 0.047), which were more different nocturnally (4.8 vs 5.2 mmol/L, p = 0.003) than diurnally (5.3 vs 5.5 mmol/L, p = 0.14). There were no differences in glucose variability between these groups. Results were independent of body mass index.

Conclusions. Among individuals without diabetes, we observed significantly different 24-hour glucose rhythms depending on calendar and biological age.

Introduction

Insulin resistance and disturbances in glucose handling are highly prevalent with increasing age, and predict the incidence of many age-related diseases, including cardiovascular events, diabetes, cancer and dementia (9; 24; 31). The insulin-IGF-1 signalling pathway is causally related to the aging process in experimental models (reviewed in (12)). In human studies, long-lived individuals often seem protected against age-associated deteriorations in glucose metabolism. For example, centenarians show an enhanced insulin sensitivity similar to middle-aged individuals (19). Moreover, middle-aged individuals with propensity for longevity have a lower prevalence of diabetes and metabolic syndrome at middle age, and - among individuals without diabetes - are more glucose tolerant and insulin sensitive compared to controls matched for age and environment (25; 33; 34). These individuals also have a lower mortality compared to their birth cohort and may be considered biologically younger than individuals from the general population of similar calendar age (27). Together, these findings suggest that preservation of efficient glucose handling might be one of the important mechanisms involved in reaching a healthy old age.

Studying glucose metabolism beyond the generally used screening methods, such as venous blood glucose measurements or glycated hemoglobin (HbA1c), often requires methods that are either non-physiological or highly invasive, such as the oral glucose tolerance test and the hyperinsulinemic euglycemic clamp study. In sharp contrast, continuous glucose monitoring is a minimally invasive method to calculate blood glucose concentration from interstitial fluid via a glucose sensor implanted in the subcutaneous tissue. As such, it enables dynamic ambulant monitoring under everyday-life conditions. Continuous glucose monitoring was introduced into clinical practice primarily to be used as a tool to lower HbA1c in patients with diabetes. Thus far, continuous glucose monitoring has rarely been used in research settings in individuals without diabetes. One recent study reports an association between increasing age and mean glucose levels obtained by continuous glucose monitoring (36). As continuous glucose monitoring generates almost three hundred glucose measurements daily, it allows for detailed characterization of 24-hour glucose rhythms, for instance by studying mean diurnal or nocturnal glucose levels, or measures of glucose variability. As yet, it is unknown how these novel parameters derived from 24-hour glucose rhythms relate to the aging process.

Here we describe associations of 24-hour glucose rhythms with calendar and biological age in individuals free of diabetes. We compare parameters of 24-hour glucose rhythms between three groups: young and middle-aged from the general population and middle-aged with propensity for longevity.

Methods

Participants

To study the relation between 24-hour glucose rhythms and biological age, we included participants with propensity for longevity from the Leiden Longevity Study (LLS). The LLS comprises 421 families, whose characteristics have been more extensively described earlier (27). Briefly, families were recruited if at least two long-lived siblings were alive and were 89 years or older for men and 91 years or older for women. There were no selection criteria on health or demographic characteristics. Because no proper controls exist for this age group, for further studies the offspring of these long-lived nonagenarians were recruited. These middle-aged individuals carry on average 50% of the genetic advantage of their long-lived parent and were shown to have a 35% lower mortality rate compared with their birth cohort (27). Their partners with whom most have had a relationship and shared environment for decades, were included as population-based environment matched middle-aged individuals.

To study the association between 24-hour glucose rhythms and calendar age, we recruited from the medical faculty a group of healthy lean volunteers to represent subjects with a low calendar age, i.e. young individuals.

In total, we included 21 young healthy individuals. From long-lived families we included 26 middle-aged individuals with propensity for longevity. We included 26 middle-aged partners of the individuals from long-lived families, plus an additional 11 lean, middle-aged individuals. All middle-aged participants had participated in another study involving 7 Tesla MRI, for which they were screened on major health criteria, and were all considered apparently healthy. All were free of diabetes. The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all subjects.

Continuous glucose monitoring

The Mini- Med® Continuous glucose monitoring System (Medtronic MiniMed Inc., Northridge, CA) was applied. A glucose sensor (Sof-Sensor®, Medtronic MiniMed Inc., Northridge, CA) was inserted into the subcutaneous abdominal fat tissue to monitor glucose levels of interstitial fluid for four consecutive days. Participants were engaged in their normal daily activities throughout the whole period. For calibration purposes, participants were instructed to perform self monitoring of capillary blood glucose four times daily using a handheld glucose meter (Bayer CONTOUR®). Participants were instructed to record at least three calibration readings the first day and four calibration readings during day two, three and four. During the study period, participants registered information on time of food intake. Results were considered valid if at least two valid calibrations were performed on day one, and at least three valid calibrations were performed on day two, three and four.

Anthropometrics

During the visit height and weight were measured, and body mass index (BMI) was calculated.

Calculations

From the recordings of the 24-hour glucose rhythms we derived parameters of mean glucose levels: the overall mean glucose levels, mean nocturnal glucose levels (03:00-06:00), mean diurnal glucose levels (06:00-00:00), minimum and maximum glucose levels and fasted glucose levels. Nocturnal hours were defined as such to optimally include resting hours without potential active or feeding behavior. Furthermore, we assessed parameters of glucose variability: range, total standard deviation (SD), mean of daily differences (MODD) and continuous overall net glycemic action over a four hour period (CONGA4). Fasted glucose was defined as the mean of the first daily self-monitored capillary blood glucose measurements before breakfast. The MODD describes the between day variability, calculated as the mean of the absolute difference of glucose values obtained at exactly the same time of day, from two consecutive days. The CONGA-n was defined as the standard deviation of the differences between the current observation and the observation n-hour previously (23). For accuracy reasons (according to manufacturers instruction), all continuous glucose values the first hours until midnight of the first day of measurement were excluded according to manufacturer's guidelines. For all analyses, SPSS software (version 17.0) was used. Comparisons between groups were calculated using ANOVA with adjustment for gender and calendar age, when

CHAPTER 5. 24-HOUR GLUCOSE RHYTHMS MARK CALENDAR AND BIOLOGICAL AGE

appropriate, and additional adjustment for body mass index. A p-value < 0.05 was considered statistically significant.

Results

Table 1 shows the baseline characteristics of young individuals, middle-aged individuals from the general population and middle-aged individuals with propensity for longevity. Mean age was slightly but non-significantly higher in the middle-aged with propensity for longevity compared to the middle-aged from the general population. In the individuals with propensity for longevity, the percentage of females was lower (39 %) than in the groups of young and middle-aged from the general population (59 % and 51 % respectively). All subsequent analyses were therefore adjusted for gender. In all groups, mean body mass index was within normal range. All groups had on average similar number of continuous glucose monitoring readings.

Table 1. Baseline characteristics

Characteristics	General population		Propensity for longevity
	Young	Middle-aged	Middle-aged
Number	21	37	26
Female sex, n (%)	13 (59.1)	19 (51.4)	10 (38.5)
Age, years	26.9 (4.3)	60.6 (7.5)	63.7 (5.8)
Weight, kg	69.9 (10.9)	76.0 (10.2)	75.5 (12.2)
Body Mass Index, kg/m²	22.6 (1.9)	24.9 (3.1)	24.2 (3.3)
Number of continuous glucose monitoring observations	959 (126)	860 (101)	825 (15)

Data represent mean with standard deviation unless stated otherwise. Number of continuous glucose monitoring observations represent number of readings per subject.

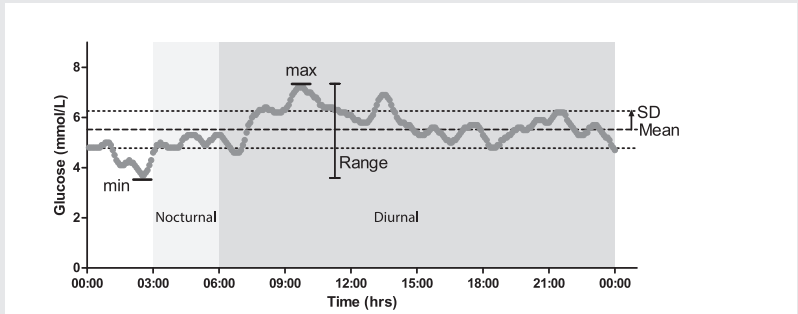

Figure 1. Representative result of 24 hours of continuous glucose monitoring in a middle-aged individual from the general population. Data represent 5-min interval glucose levels. Nocturnal was defined as time between 03:00 and 06:00 hrs. Diurnal was defined as time between 06:00 and 00:00 hrs.

Figure 1 shows a representative one-day example glucose rhythm of a middle-aged individual from the general population with depiction of several calculated parameters of mean glucose levels and variability.

To assess which parameters of 24-hour glucose rhythm associated with calendar age, we first compared different parameters of 24-hour glucose rhythms between young and middle-aged from the general population. Visual inspection of figure 2a reveals that compared to the group of young from the general population, the middle-aged had lower mean glucose levels during all time points. Table 2 depicts the differences in parameters of glucose levels (mean, diurnal, and nocturnal) as well as parameters of glucose variability such as range, total standard deviation, mean of daily differences and continuous net glycemic action. After adjustment for gender, middle-aged individuals from the general population showed higher mean glucose levels compared to young (5.3 vs 4.7 mmol/L, p <0.001), as well as higher diurnal (5.5 vs 4.9 mmol/L, p <0.001) and higher nocturnal glucose levels (5.2 vs 4.8 mmol/L, p = 0.002). Furthermore, middle-aged individuals had higher fasted glucose (5.2 vs 4.7 mmol/L, p=0.001) and a higher maximum glucose (7.8 vs 6.9 mmol/L, p = 0.003), but similar minimum glucose levels. Glucose variability was higher in middle-aged individuals as measured by total standard deviation (0.70 vs 0.57 mmol/L, p = 0.025), continuous net glycemic action over a 4-hour period (0.90 vs 0.70 mmol/L, p = 0.009) and mean of daily

differences, although of borderline statistical significance (0.73 vs 0.61 mmol/L, p=0.066).

To test whether 24-hour glucose rhythms mark differences in biological age, we compared parameters of 24-hour glucose rhythms between middle-aged from the general population with middle-aged with propensity for longevity. Visual inspection of the cumulative curves in figure 2b reveals that middle-aged with the propensity for longevity showed lower glucose levels during nocturnal hours. Table 2 shows the

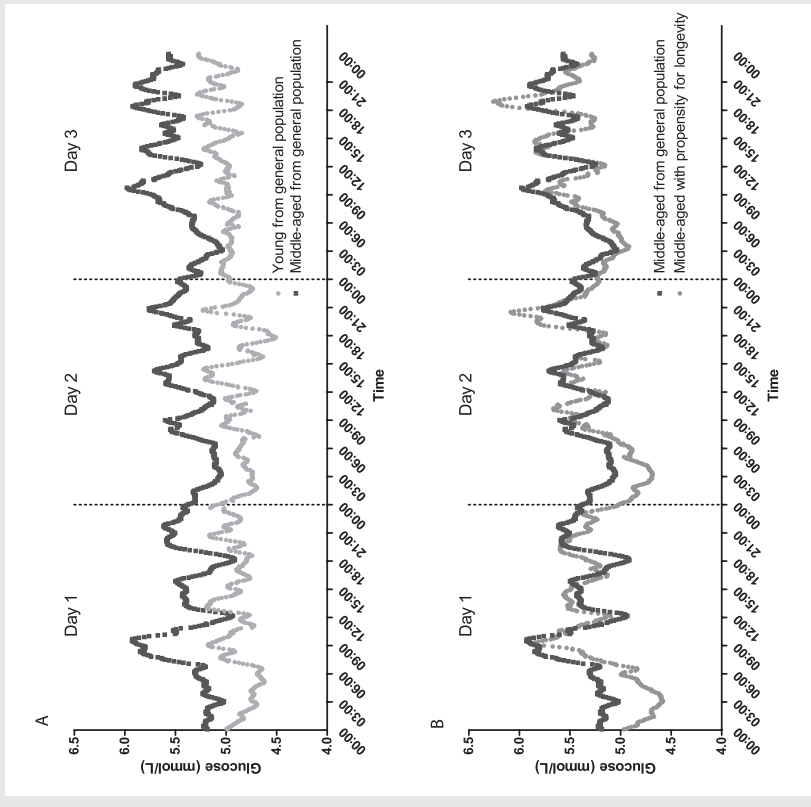

Figure 2 A-B. Mean glucose rhythms during 72 hours of groups of a) young (n=21) vs. middle-aged from the general population (n=37), and b) middle-aged from the general population vs. middle-aged with propensity for longevity (n=26). Data points represent mean 5-min. intervals of glucose levels.

Table 2. Differences in parameters of 24-hour glucose rhythms between study groups of different calendar and biological age

	General population		Propensity for longevity	P-value*	P-value**
	Young n = 21	Middle-aged n= 37	Middle-aged n = 26		
Glucose levels					
Overall mean glucose	4.91	5.41	5.19	**<0.001**	**0.047**
95% C.I.	4.73 - 5.07	5.28 - 5.54	5.03 - 5.34		
Diurnal glucose (06:00 - 00:00 hrs)	4.93	5.48	5.32	**<0.001**	0.14
95% C.I.	4.75 - 5.11	5.35 - 5.61	5.16 - 5.48		
Nocturnal glucose (03:00 - 06:00 hrs)	4.80	5.17	4.80	**0.002**	**0.004**
95% C.I.	4.61 – 5.00	5.03- 5.32	4.62 - 4.97		
Mean fasted glucose	4.73	5.15	4.87	**0.001**	**0.021**
95% C.I.	4.52 - 4.95	5.00 - 5.31	4.68 - 5.06		
Glucose variability					
Minimum	3.45	3.59	3.50	0.37	0.72
95% C.I.	3.10 - 3.70	3.40 - 3.78	3.27 - 3.72		
Maximum	6.89	7.80	7.86	**0.003**	0.87
95% C.I.	5.40 - 7.38	7.43 - 8.16	7.41 - 8.30		
Range	3.44	4.21	4.36	**0.045**	0.79
95% C.I.	2.82 - 4.06	3.74 - 4.67	3.80 - 4.92		
Total standard deviation (SDt)#	0.57	0.70	0.72	**0.025**	0.78
95% C.I.	0.49 - 0.66	0.62 - 0.78	0.63 - 0.82		
Continuous net glycemic action (CONGA4)#	0.70	0.90	0.90	**0.009**	1.00
95% C.I.	0.60 - 0.81	0.80 - 1.01	0.79 - 1.04		
Mean of daily differences (MODD) #	0.61	0.73	0.71	0.066	0.73
95% C.I.	0.53 - 0.71	0.63 - 0.82	0.62 - 0.81		

*Data represent means with 95% confidence intervals in mmol/L. # geometric means due to skewness of data. * P-value for difference between young and middle-aged from general population, with adjustment for gender ** P-value for difference between middle-aged from general population and middle-aged with propensity for longevity, with adjustment for age and gender.*

differences in these parameters after adjustment for age and gender. The middle-aged with propensity for longevity showed significantly lower mean glucose levels (5.2 vs 5.4 mmol/L, p = 0.047) which were driven by a significantly lower nocturnal glucose concentration (4.8 vs 5.2 mmol/L, p = 0.003), and less outspoken differences in diurnal glucose levels (5.3 vs 5.5 mmol/L, p = 0.14), in comparison to the middle-aged from the general population. Fasted glucose levels were also significantly lower in the middle-aged with the propensity for longevity (4.9 vs 5.2 mmol/L, p = 0.021). No differences were found in any parameters of glucose variability between the groups.

We then explored whether the observed differences between groups could be explained by body mass index. First, after adjustment for age and gender, we observed no association between body mass index and parameters of 24-hour glucose rhythms among the young and middle-aged from the general population. Next, we repeated the analyses in table 2 now adjusting for body mass index. Between the young and middle-aged from the general population, we found that the differences in mean glucose remained similar (4.9 vs 5.4 mmol/L, p <0.001), as did the other differences in parameters of 24-hour glucose rhythms. Finally, we assessed whether the difference in nocturnal glucose between groups of middle-aged with propensity for longevity and the general population was dependent on body mass index. After adjustment for body mass index, the differences in nocturnal glucose between middle-aged with propensity for longevity and middle-aged from the general population remained similar (4.8 vs 5.2 mmol/L, p = 0.004). As sensitivity analysis, further exclusion of two participants of the middle-aged from the general population who reached the criteria for obesity (body mass index > 30), did also not materially change this difference (4.8 vs 5.2 mmol/L, p = 0.006).

Discussion

The main findings of this study are twofold. First, we observed a significant difference in most parameters of 24-hour glucose rhythms with different calendar age. Second, we observed lower mean glucose levels driven by nocturnal glucose levels in individuals with propensity for longevity compared to middle-aged individuals from the general population. Our findings may provide new insights in early markers of decrease in glucose handling and diabetes in relation to chronological and biological age.

Calendar age associated with all parameters of glucose levels and its variability in our study. Most previous studies using continuous glucose monitoring have focussed on the effect of glucose monitoring on therapeutic outcomes, such as the effect on HbA1c in type 1 or type 2 diabetics (11; 20). Others have assessed associations between glucose rhythms and disease in patients with diabetes (4; 6; 17; 22; 29), or focused on the role of glucose variability in patients in an intensive care unit (7; 8; 13). Few studies have assessed glucose rhythms in non-diabetic individuals, and data on the relationship between aging and parameters of continuous glucose monitoring are scarce. In a Chinese study in over four hundred healthy lean individuals, 24-hour mean blood glucose levels were found to be higher with increasing age (36), and total standard deviation was also slightly higher with increasing age (37). However, other parameters were not reported in this study. A study in eighteen healthy subjects aged between 20 and 40 years old also found an increased mean blood glucose levels with increasing age (5). In the subset of non-diabetic subjects from the Juvenile Diabetes Study this relationship was not seen (10). However, in this particular study most participants were child or adolescent. In our study, in which we systematically studied 10 different parameters from 24-hour glucose rhythms, we found higher overall mean glucose levels and standard deviation in middle-aged compared to young individuals. Moreover, we found that these differences were found in both diurnal and nocturnal glucose levels, and in maximum, but not minimum, glucose levels. These findings suggest that, with increasing calendar age in individuals without diabetes, there is a deterioration of overall glucose handling. This could be reflected by gradual loss of both the glucose ‘thermostat’, i.e. the low glucose threshold the body aims at keeping stable as well as the variability around this threshold, which could reflect a loss of ability to adequately respond to stimuli like the insulin response upon meals.

Earlier, in the Leiden Longevity study, we found differences in non-fasting glucose levels, glucose tolerance and insulin sensitivity in offspring of nonagenarian sibling compared to controls (25; 33; 34), reflecting differences in insulin-dependent glucose metabolism. Our main finding now is a difference in a fasted state at night time during sleep which reflects a non-insulin dependent glucose level. Despite prolonged fasting during night time, earlier studies show that nocturnal glucose levels remain stable or fall only minimally, contrasting with day time fasting during which glucose levels fall more prominently (3). This shows that counterregulatory mechanisms operate during the night to prevent glucose levels from falling. One of these is growth hormone, the production of which is induced during sleep onset.

This, in combination with decreased glucose utilization, results in increasing glucose levels during the beginning of the night (3). In the second half of the night, glucose levels return to baseline, potentially due to attenuation of the inhibiting effect of growth hormone on tissue glucose uptake (16), increased glucose utilization during REM-sleep and waking periods (26), or as a delayed response to low cortisol levels in the late evening and early night (21). Potentially, alterations in sleeping patterns in hormonal regulation might explain the lower nocturnal glucose in the end of the night related to biological age in our study. Furthermore, individuals with propensity for longevity might represent a rare phenotype that is specifically characterised by overall enhanced glucose metabolism through mechanisms that differ from the etiology of metabolic disturbances in the general population, such as physical inactivity or obesity. Earlier we found that neither the genetic burden of disease susceptibility genes in general (1), nor diabetes risk alleles (18), explained the observed differences in glucose metabolism. Additionally, middle-aged individuals with propensity for longevity have shown not to differ in environmental factors related to metabolism, such as body composition or physical activity or other lifestyle parameters such as smoking, compared to controls from the general population (25). Speculating further, perhaps the explanation of our findings could be found in other mammals with the longevity phenotype, which have shown for example enhanced fatty acid over glucose oxidation, lower core body temperature and higher metabolic rate (32). In line with these findings we reported in the same study population that the middle-aged individuals had lower levels of intramyocellular lipid content, which may be the result of decreased muscle fatty acid oxidation (35). Further research should investigate whether differences in circadian hormone rhythms, sleep quality or differences in metabolic rate can explain our findings of nocturnal differences in glucose related to familial longevity.

In this study we aimed to assess glucose rhythms in a setting that optimally resembles daily life. Therefore, we did not record, or standardize on, food intake or physical activity in detail. Based on these methods, our results are of descriptive nature. Physical activity is a powerful modulator of glucose excursions, also on very short term (14; 15). Therefore, differences in physical activity patterns with age might be an important lifestyle factor that could have modulated our findings. We aimed to minimalize the influence of differences in body fat on the observed relations between glucose rhythms and age by further adjusting for residual differences in BMI, but we cannot rule out the possibility that differences in other body fat parameters could

account for the observed results. Further study should elucidate the role of physical activity and detailed body composition in 24-hour glucose rhythms. In this study glucose variability was expressed using SD, CONGA4 and MODD. We do not report on another commonly used parameter for glucose variability, mean amplitude of glycemic excursion (MAGE)(28). MAGE was not specifically designed for CGM and uses arbitrary cut-off points, which might wrongfully ignore smaller glucose excursions. Other measures that do take into account all glucose excursions, such as CONGA and SD, show strong correlations ($r > 0.85$) with MAGE (2; 23; 30), indicating that reporting MAGE does not yield additive information. Furthermore, SD and CONGA are less difficult to compute with the large number of data obtained with CGM and easier to explain to patients and colleague. For these reasons, we believe MAGE not to be an important parameter for the analysis of CGM and chose not to report on it in the current study. A limitation of CGM is the time lag between blood glucose and interstitial glucose levels, estimated to be within 5 and 15 minutes, which could lead to inaccuracies between CGM readings and actual blood glucose levels. Furthermore, calibration should ideally be performed when glucose levels are steady state, which we have tried to optimize by instructing participants to measure capillary glucose before meals.

Strength of our study is that we have used a systematic approach to analyse different parameters of 24-hour glucose rhythms, yielding a detailed analysis of mean glucose levels and variability. Furthermore, we used a unique cohort to study determinants of familial longevity using the Leiden Longevity Study. Using married couples, we have included individuals different in propensity for longevity but similar in lifestyle factors. Furthermore, different subcohorts from this study population have consistently shown better glucose handling in middle-aged offspring of long-lived families compared to their partners, which strengthens the reliability of our findings of better glucose handling in relation to familial longevity and biological age. Moreover, we are, to our knowledge, the first to have used continuous glucose monitoring to study glucose rhythms in relation to familial longevity.

We have successfully used continuous glucose monitoring in healthy individuals to describe associations between glucose rhythms and calendar and biological age. Further studies should use continuous glucose monitoring to study glucose metabolism in normal daily life. Moreover, parameters of continuous glucose monitoring should be used to monitor the effects of interventions aimed at improving

glucose metabolism at a larger scale and further unravel the associations between glucose metabolism and healthy aging.

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