



Conference on ‘Roles of sleep and circadian rhythms in the origin and nutritional management of obesity and metabolic disease’ Symposium 4: Influence of lifestyle and genetics

Circadian rhythms, food timing and obesity

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It is known that our physiology changes throughout the day and that several physiological hormones display circadian rhythmicity. The alteration of this normal pattern is called chronodisruption (CD). In recent years, it has been demonstrated that CD is related to obesity. Although several factors may be causing CD, one important aspect to consider is the failure in our internal clock. Indeed, studies performed in mutant animals have demonstrated that mutations in clock genes are related to obesity. In human subjects, mutations are rare (<1 % of the population). Nevertheless, it is rather common to have genetic variations in one SNP, which underlie differences in our vulnerability to disease. Several SNP in clock genes are related to obesity and weight loss. Taking into account that genetics is behind CD, as has already been demonstrated in twins’ models, the question is: Are we predestinated? We will see along these lines that nutrigenetics and epigenetics answer: ‘No, we are not predestinated’. Through nutrigenetics we know that our behaviours may interact with our genes and may decrease the deleterious effect of one specific risk variant. From epigenetics the message is even more positive: it is demonstrated that by changing our behaviours we can change our genome. Herein, we propose modifying ‘what, how, and when we eat’ as an effective tool to decrease our genetic risk, and as a consequence to diminish CD and decrease obesity. This is a novel and very promising area in obesity prevention and treatment.

Nutrigenetic: Circadian rhythm: Food timing: Obesity

Our physiology changes during the day. It is well known that several hormones that are related to obesity, such as cortisol, leptin and adiponectin, among others, display circadian rhythmicity^(1–3). The alteration of this normal pattern is called chronodisruption (CD), and it is related to several disturbances in many systems and organs of our body. In recent years, it has been demonstrated that CD is related to obesity.

CD or circadian disruption can be defined as ‘a serious disruption of the internal temporal order of the biochemical, physiological and behavioural circadian rhythms’⁽⁴⁾. In our modern society, CD may be produced by several environmental factors, or external situations that are

relatively common in the current society such as jet lag, shift work, night light pollution or overnight recreational activities (social jet lag)⁽⁵⁾. Other factors are internal, and may produce CD by alteration of the core machinery of the molecular circadian clock. Brain- and muscle ANRT-like protein-1 (BMAL1), Period 2 (PER2) and circadian locomotor output cycles kaput (CLOCK), among others clock proteins, have a specific role in our physiology as well as in the circadian molecular clock. Indeed, several studies performed in mutant animals have demonstrated that mutations in clock genes are related to obesity, ageing and other metabolic alterations implicated in several chronic diseases^(6,7). For example,

Abbreviations: BMAL1, brain- and muscle ANRT-like protein-1; CD, chronodisruption; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; MetS, metabolic syndrome; PER2, Period 2; WT, wrist temperature.

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studies performed in experimental animals have shown that those animals that have mutations in *Clock* are more obese and display metabolic disturbances⁽⁶⁾.

In human subjects, mutations are very rare. Nevertheless, it is rather common to have genetic variations in one single nucleotide (SNP) that underlie differences in our vulnerability to disease. Regarding the circadian machinery, it is well known that several genetic variants are related to obesity, obesogenic behaviours and also to the effectiveness of the treatment in weight-loss programmes.

It is relevant to consider that clock genes are not only associated with obesity, but they also may interact with several obesogenic behaviours for obesity or weight-control parameters. One clear example is the interaction between *CLOCK 3111T/C* and emotional eating behaviours⁽⁸⁾; Our results demonstrate that during a dietary treatment to lose weight, those subjects who were 'very emotional eaters' and also carriers of the risk allele C in *CLOCK 3111T/C* lost less weight, in comparison with (a) C carriers that were non-emotional eaters, and (b) TT carriers independently of their emotional status. Many other examples of interactions between clock genes and several behaviours will be explained in the following text. Thus, through nutrigenetics we know that our behaviours may interact with our genes and may decrease the deleterious effect of one specific risk variant; in other words, we can change our obesity predisposition and, although we are not able to change our genetic code, we can definitely change our behaviours.

From epigenetics, the message is even more positive: it is demonstrated that by changing our behaviour we can even change our genome. For example, we have demonstrated in *CLOCK* that DNA methylation levels at different CpG sites of *CLOCK* are higher in obese than in non-obese women, and these methylation levels were associated with several obesogenic behaviours such as snacking frequently, eating when bored or eating from large packages. Therefore, we presume from these data that, through some small but stable changes in our eating behaviours, we are able to change the DNA structure as a consequence of gene expression and, more importantly, we can change our destiny.

Chronodisruption and failures in the central clock

It has been previously described that CD can be the result of alterations in circadian system at different levels. Impairments can be present in the inputs or outputs to the circadian central clock located in the suprachiasmatic nucleus, but also in the central clock. With regard to the inputs, failures appear due to different circumstances, such as: (1) no changes between day/night synchronising agents, such as light/dark, timing of food intake (eating/fasting) or programmed exercise (activity/rest); (2) different periods or unusual phasing of synchronising agents, for example: light at night, nocturnal feeding or physical activity; and (3) shifts in the time provided by zeitgebers (i.e. jet lag, shift work). Outputs of the central pacemaker may also be altered: suppression of melatonin at night or

loss of glucocorticoids rhythm like cortisol. Other problems in the central clock can result for example from the desynchronisation between the central pacemaker and peripheral oscillators or alterations in the so-called clock genes.

Nowadays we know that the internal clock regulates our physiological changes throughout the day. This clock works as a result of the expression of several clock genes that may activate and deactivate the clock to display a general pattern of 24 h. These clock genes can be classified into two groups: positive and negative. *CLOCK* and *BMAL1* act as positive genes (they activate this clock) and are responsible for the synthesis of two transcriptional factors which, after dimerisation (*CLOCK*–*BMAL1*), induce the expression of negative genes; these are *PER* and cryptochrome (*CRY*), together with *nuclear receptor subfamily 1, group D, member 2 alpha* (a transcription factor). These negative elements, after dimerisation (*PER*–*CRY*), undergo a nuclear translocation and act as suppressors of *CLOCK* and *BMAL1* expression, consequently slowing and stopping the clock.

The levels of positive and negative elements oscillate in antiphase generating circadian rhythms (with a period of approximately 24 h) in suprachiasmatic nucleus *in vitro*⁽⁹⁾. Although the circadian system is mainly formed by the central pacemaker in suprachiasmatic nucleus, it is known since 2001 that this central clock, in turn, synchronises the activity of several peripheral clocks present in our organs and tissues, such as heart, lung, liver, oral mucosa, pancreas and adipose tissue, among others, by a cyclical secretion of hormones and the activation of the autonomic nervous system⁽¹⁰⁾.

Failures in the central clock: mutations in experimental animals

There are many factors, which may affect the circadian system at different levels. In relation to clock genes, numerous studies performed in experimental animals with mutations in clock genes have proved that there is a relation between these mutations and further failures in circadian system and illness. In this sense, animals with mutations in clock genes show a higher risk of developing certain diseases, such as CVD, cancer and obesity⁽⁵⁾.

One of the first studies which showed the effect of genetic mutations on chronic illnesses was conducted in 2005 by Turek *et al.*⁽⁶⁾ This study proved that homozygous *Clock* mutant mice were hyperphagic and obese, displayed adipocyte hypertrophy and developed metabolic syndrome (MetS). However, there is already controversy. Results have not proved to be consistent across different experiments. For example, Kennaway *et al.* showed that *Clock* (*Delta19*) mutation in mice did not cause obesity; by contrast, the authors found reduced plasma-free fatty acids and increased insulin sensitivity, together with increased plasma adiponectin, a protective anti-inflammatory cytokine⁽⁷⁾.

Further studies have shown that mutations in different clock genes are related to an increased risk of developing certain diseases, such as premature ageing, as has been

demonstrated by Kondratov *et al.* In this study, *Bmal1* ($^{-/-}$) mice had reduced lifespan (mean lifespan of knock-out animals was 37 v. 120 weeks for wild-type animals) and displayed various symptoms of premature ageing, including sarcopenia, alteration of the percentage of lymphocytes and impaired vision among others⁽¹¹⁾.

Genetic variations of clock genes in human subjects *Clock genes associate with obesity in human subjects.*

Mutations are very rare in human subjects. However, it is rather common to have genetic variations in one SNP at some specific position of the genome, each of these variations being present in more than 1 % of the general population. These genetic variations underlie differences in our vulnerability to several diseases such as obesity.

In this sense, the work performed by Sookoian *et al.* in 2008 was the first to demonstrate that several variants at *CLOCK* were associated with obesity, especially with abdominal obesity⁽¹²⁾. In addition, in the same year, Scott *et al.* confirmed these results by showing that *CLOCK* could play a relevant role in the development of MetS, type 2 diabetes and CVD⁽¹³⁾.

Subsequently, our research group replicated the results of Sookoian and Scott and also demonstrated that several genetic variants in clock genes were related to obesity and related diseases such as MetS. For example, *CLOCK* SNP (rs3749474, rs4580704 and rs1801260 (3111T > C)) were associated with BMI, energy intake and different variables related to obesity⁽¹⁴⁾. In fact, our results showed that carriers of the minor alleles consumed more energy⁽¹⁵⁾ (1046 kJ (250 kcal)/d, ate more fat (5–10 g), and were more obese (3 kg body weight) for rs3749474, rs1801260 (3111T > C)). Conversely, minor allele carriers (A) of *CLOCK* rs4580704 eat less energy and fat, were less obese and showed a decreased risk of developing diabetes (31 % lower) and hypertension (46 % lower) than non-carriers⁽¹⁴⁾. Besides, one genetic variation in *CLOCK* rs1801260 (3111T > C) was associated with increased obesity and with less weight loss⁽¹⁶⁾. Moreover, the proportion of subjects who slept < 6 h/d was higher among subjects with the C minor variant of this polymorphism than non-carriers (59 v. 41 %)⁽¹⁷⁾. Some of these associations are functionally explained by the presence of a polymorphism involving a change in the structure of the mRNA leading to a change in gene expression; this is the case of the *CLOCK* polymorphism rs3749474⁽¹⁵⁾. Most of these novel results were replicated in two different populations (Mediterranean and North American) with different genetic backgrounds and dietary habits, which emphasise the robustness of the findings.

Other results from our group also showed that different genetic variants of the clock such as *nuclear receptor subfamily 1, group D, member 2 alpha* rs2314339 were also associated with obesity. Once more, these results were replicated in two independent populations: Mediterranean and North American. This gene is the major regulator of rhythmic *BMAL1* transcription and it is considered to be the molecular link between the positive elements of the clock (*CLOCK* and *BMAL1*)

and the negative (*PER* and *CRY*). One specific aspect in this association was that *nuclear receptor subfamily 1, group D, member 2 alpha* rs2314339 is associated with obesity through a decrease in physical activity⁽¹⁸⁾, differently to other clock genetic variants, which were associated with obesity through changes in dietary intake. Indeed, our findings are consistent with those obtained in experimental animals, which demonstrated that mutants *RevErba* $^{-/-}$ mice displayed a significantly lower spontaneous locomotor activity than their wild-type littermates⁽¹⁹⁾.

Our research group has also found significant associations between clock genes from the negative element of our circadian system and several obesogenic behaviours. In this regard, a key clock gene is *PER2*. A missense mutation in human *PER2* has been linked to several psychological alterations, such as seasonal variations in mood and behaviour, and winter depression⁽²⁰⁾. Our group was the first one to demonstrate that some specific *PER2* SNP (rs2304672C > G and rs4663302C > T) were associated with abdominal obesity⁽²¹⁾. In particular, *PER2* rs2304672C > G minor allele carriers G (6 % of the population) displayed several obesogenic behaviours such as an increased attrition of the weight-loss treatment, increased frequency of snacking, stress while dieting, eating while bored and skipping breakfast, when compared with non-carriers C⁽²¹⁾. It is impressive to observe how a small change in our genome (a cytosine for a guanine) may produce such a big variation in the mRNA structure and, as a consequence, changes in our gene expression.

Clock genes interact with several behaviours for obesity in human subjects. Clock genes may also interact with behaviours for obesity. This is the case of those behaviours directly related to emotional eating. For example, seeking refuge in food (especially high-energy foods) is a very common strategy to reduce anxiety, sadness and negative emotions that occur when following a long-term diet or due to difficult circumstances of our daily lives. Our results demonstrated that those participants of our weight-loss programme who were carriers of the risk allele C at *CLOCK* 3111T > C and displayed emotional eating behaviours more frequently, had more difficulties to lose weight during the treatment. Interestingly, C carriers who did not display emotional behaviours, in spite of being potentially at risk due to their genetic background, showed a similar weight loss as TT carriers (protective allele). These results are encouraging because they show that, by changing 'how' we eat, we can reduce or even eliminate the deleterious effect of a genetic variant.

In this sense, our research group has developed a ten-item questionnaire called Emotional-Eater-Questionnaire to be used in clinical practice, which classifies obese individuals as a function of the relation between food intake and emotions. The questionnaire allows classification of individuals as non-emotional eaters (emotions have little or nothing to do with their eating behaviour) and emotional eaters (feelings and emotions affect their eating as a response to negative emotions, such as anxiety, depression, anger and loneliness).

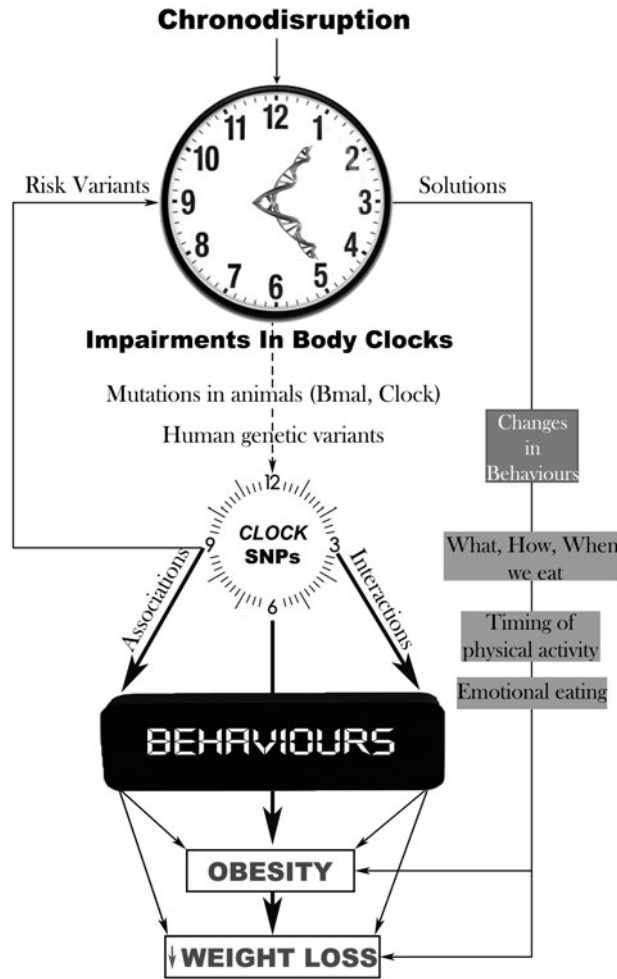


Fig. 1. Represents how several impairments in the central clock are related directly with obesity. In experimental animals, these impairments are due to mutations; however in human subjects, mutations are rare and these impairments are caused by human genetic variants. Thus, these human genetic variants may be associated directly with obesity or through different behaviours. Moreover, these genetic variants may interact with different behaviours to influence obesity and weight loss. We can consider several solutions: (a) changing our behaviours, these changes are directly related to a decrease in obesity and an increase in weight loss, or (b) through genetics by changes in our behaviours that may interact with the SNP to produce a decrease in obesity and weight loss.

Out of a total population of 1500 subjects, we found that 60 % were emotional eaters, 40 % were minor C allele carriers and 30 % were both C carriers and emotional eaters. Considering these results, it may be useful to develop behavioural and cognitive programmes aiming to reduce the frequency of emotional eating episodes, particularly for the 30 % who carry the risk variant C. These results are encouraging and should be used in clinical practice.

Clock genes are related to weight loss

Genetics and weight loss. One of the main problems in weight-loss treatments is the dramatic inter-individual variability in response to the treatment. It is believed that an elucidation of the genetic component will help to predict weight-loss effectiveness. Some studies carried out in monozygotic twins analysed genetic factors for effectiveness of weight-loss programmes, other studies emphasised the

familial aggregation in their ability for losing weight; and the role that parental obesity plays in this matter.

However, most of the prior genetic studies focused on candidate obesity-related genes (NUGENOB) found little association between genetics and weight loss, except for some cases (PPARG)⁽²²⁾. Notably, no association with weight loss was observed for thirteen obesity risk SNP from the genome-wide association study⁽²³⁾, suggesting that the genetic architecture of obesity and weight-loss effectiveness may differ, or that gene-environment effects need to be considered.

Surprisingly, clock genes are related to weight loss. In this sense, our most relevant results show that there is an association between a *CLOCK* 3111T > C (rs1801260) and weight-loss effectiveness⁽¹⁶⁾ (Fig. 1). This study demonstrated that C carriers were more resistant to weight lost than TT homozygotes, when these individuals were subjected to an energy-restricted diet⁽¹⁶⁾. These data suggest that clock SNP may predict weight loss in response to a low-energy diet.



We also reported that *SIRT1* and *CLOCK* 3111T>C combined genotype was associated with a particular chronotype and with weight-loss resistance in a behavioural therapy treatment for obesity⁽²⁴⁾. In this work, subjects carrying minor alleles at *SIRT1* and *CLOCK* displayed a significantly lower total weight loss and lower weekly weight-loss rate as compared with homozygotes for both major alleles.

Main barriers to lose weight (environmental factors).

It is classically known that weight-loss effectiveness is highly related to environmental factors⁽²⁵⁾. In a study performed in 1400 obese subjects from a Mediterranean area in southeast Spain who attended a weight-reduction programme, those subjects who lost less weight during the treatment had more obstacles and higher 'barriers to lose weight' score (double) than those who achieved their goal. One of the main barriers was the lack of motivation. In fact, those patients who did not achieve their weight goal (loss of at least 10 % of their initial weight) displayed 83 % more predisposition to lose their motivation than those who achieved it. These patients were also predisposed to suffer other barriers related to emotional eating, such as stress-related diet, thinking in 'black or white terms' or eating while bored⁽²⁵⁾.

Genetics associates with obesogenic behaviours to modulate total weight loss. Furthermore, we have discovered how certain polymorphisms associate with different environmental factors to modulate total weight loss⁽⁸⁾. This is the case of *CLOCK* 3111T>C (rs1801260) SNP. The present author group have shown that minor allele C carriers of *CLOCK* 3111T>C were more evening type, had less healthy dietary habits and tended to sleep less than T carriers⁽⁸⁾. Moreover, the combination of *SIRT1* and *CLOCK* 3111T>C was also associated with evening preference, with less adherence to Mediterranean food intake patterns and with higher plasma levels of ghrelin (hunger hormone)⁽²⁴⁾.

Currently, one of the greatest challenges in weight-loss treatments is to detect good predictors of success. The analysis of genes, dietary intake and behavioural factors could be used as a tool to increase the success of such treatments.

Genetics variants in clock associate with alterations in circadian rhythmicity (chronodisruption), and these alterations may predict a lower effectiveness of the treatment. In order to achieve a better understanding of the relationship between circadian rhythms and obesity, we evaluated changes in circadian rhythmicity with *CLOCK* 3111T>C SNP in overweight women, by recording continuously body peripheral temperature (wrist temperature (WT)), actimetry and position during 1 week. These variables have been described as good markers of CD⁽²⁶⁾. Our results showed that risk carriers (C) displayed significant circadian abnormalities such as: (a) lower amplitude, (b) greater fragmentation of the rhythm, (c) less stable circadian pattern and (d) significantly decreased circadian function, as was shown after assessing by the circadian function index. C subjects were also less active, started their activities later in the morning and were sleepier during the day, showing a delayed acrophase that characterises the evening-type subject⁽²⁶⁾.

In a subsequent study, recording the same markers of CD (WT, actimetry and position), we also demonstrated that alterations in circadian rhythms (CD) could be good predictors of a lower effectiveness of weight-loss treatments. Our results showed that those subjects who were low responders in the treatment displayed a more flattened pattern of WT characterised by lower amplitude, higher intradaily variability and deteriorated circadian functionality index when compared with the individuals who had a greater response to the treatment⁽²⁷⁾. This work supports the hypothesis that analysing the circadian rhythms of the subjects at the beginning of the treatment could be useful to predict future weight loss⁽²⁷⁾.

Genetics and chronobiology (twins study)

After having proved that CD and several genetic variants in clock are related to obesity⁽¹⁴⁾, the next step was to determine to what extent CD is determined by our genetics or if it is mainly driven by environmental factors. For this purpose, in 2013 we determined the circadian system heritability as assessed by WT, using classical twin models⁽²⁸⁾. In twin studies, we can distinguish between genetic and environmental variations because monozygotic twins share 100 % of their genes and dizygotic twins share, on average, 50 % of their genetic makeup⁽²⁹⁾.

The sample selected for the study comprised fifty-three pairs of female twins: twenty-eight monozygotic and twenty-five dizygotic, who carried a sensor to measure WT for one week^(28,30). When we analysed the average weekly temperature waves, we realised that in monozygotic twins the patterns were very similar between sisters, not only in those sisters who displayed a regular circadian pattern, but also in those who had an irregular pattern with more flattened curves. However, among dizygotic twins, patterns were rather different between sisters. These first observations demonstrated that genetics play an important role in the circadian rhythm of peripheral body temperature and also in CD.

Further results showed that the individual chronotype, i.e. to be morning or evening type, was mainly driven by genetic factors (about 70 %). Nevertheless, when analysing other characteristics of the WT rhythm as the amplitude of the rhythm and interdaily stability, which play an important role in the CD and obesity; the environmental component was stronger than the genetic. Environmental factors are those external influences that can be controlled and modified by the intervention; for example, changing the subject's behaviours such as the timing of food intake and of physical activity or bedtime. Therefore, we concluded that in the clinical practice, it could be useful to focus efforts in modifying these environmental parameters in order to improve the circadian patient's health.

Nutrigenetics says: our genome may interact with our behaviours

Over the course of many years, researchers have been focused on the search for potential interactions between

Table 1. Advices to prevent genetic and behavioural association/interactions for obesity that may be crucial for genetically informed personalised nutrition in obesity treatment.

1. Evidences in nutrigenetic			
SNP	Evidence	Recommendation	References
1.A. Associations			
<i>CLOCK</i> SNP rs3749474 rs4580704 rs1801260 (3111T > C)	Associated with BMI, energy intake, and different variables related to obesity (minor alleles carriers ate more, ate more fat and were more obese)	In minor alleles carriers: - Decrease energy intake - Decrease total fat intake	(14)
<i>CLOCK</i> rs1801260 (3111T > C)	Associated with weight loss (C allele carriers were more resistant to weight loss) And were more evening type, slept less and had less adherence to the Mediterranean diet	In C allele carriers: - Sleep at least 8 h/d - Get up earlier in the morning - Go to bed earlier - Follow Mediterranean diet patterns	(8)
<i>REVERBα</i> rs2314339	Associated with obesity due to a decrease in physical activity, this physical activity was performed in the afternoon	In A allele carriers: - Increase physical activity - Perform physical activity in the morning	(18)
<i>PER2</i> rs2304672	Associated with several obesogenic behaviours such as attrition of weight loss treatment, snacking, stress while dieting, eating while bored and skipping breakfast (allele minor carriers)	In G allele carriers: - Develop a stronger follow up plan during dietary therapy - Avoid snacking - Avoid being around food when bored - Try having always breakfast	(21)
<i>SIRT1</i> and <i>CLOCK</i> 3111T > C combined genotype	Associated with evening preference, with fewer adherences to Mediterranean food intake patterns and with higher plasma levels of ghrelin in subjects carrying minor alleles of both SNP	In minor alleles carriers of both SNP: - Get up earlier in the morning - Follow Mediterranean diet patterns - Try to have five meals/d to prevent hunger	(24)
1.B. Interactions			
<i>CLOCK</i> rs1801260 (3111T > C)	Interact with: 1. Emotions for obesity (among C allele carriers, 'emotional eaters' lost significantly less weight than 'non-emotional eaters'). 2. SFA for waist circumference (among C allele carriers when the SFA energy intake was more than 11.8 % had higher waist circumference)	Among C allele carriers the 'emotional eaters': - Develop a stronger follow up plan during dietary therapy - Try to have SFA energy intake lower than 11.8 %	(8,14)
<i>CLOCK</i> rs4580704	Interact with MUFA intake (% of energy) for glucose plasma values and HOMA (with a MUFA intake was higher than 13.2 %, minor allele carriers, had lower glucose plasma concentrations and HOMA than non-carriers)	In CC carriers: – Increase the intake of olive oil	(14)
<i>CRY1</i> rs2287161	Interact with carbohydrate intake for glucose metabolism (among CC carriers when they ate high values of carbohydrates, their insulin resistance (HOMA) was higher than G carriers (GG + GC))	Among CC carriers: – Try to eat less carbohydrate from total energy intake	(31)
<i>MTNR1B</i> rs10830963	Interact with melatonin intake for glucose resistance	In GG carriers: – Avoid consuming food when melatonin levels are elevated	(33)
Evidences in epigenetic			
CpG sites of <i>CLOCK</i>	Eating behaviours, such as a high frequency of snacking, eating quickly, eating when bored or eating from large packages, were all positively associated with the methylation levels of <i>CLOCK</i> CpG 1	Try to avoid these eating behaviours: snacking, eating slowly, eating when bored and or eating from large packages	(1)
CpG sites of <i>BMAL1</i>	Evening type people presented more epigenetics modifications (high methylations in the CpG 5–9) due to weight loss intervention than morning types	Get up earlier in the morning	(36)

HOMA, homeostatic model assessment.

genetic and environmental behaviours, such as nutrition, for various physiological and pathophysiological aspects. In fact, over the last few years it has been demonstrated that different SNP in our circadian machinery interact

with dietary intake and several behaviours for obesity and for other metabolic risk (MetS)-related variables^(8,14,31).

In this sense, in 2009 we revealed several interactions between *CLOCK* variants and dietary intakes for different

MetS traits. We showed a significant interaction between *CLOCK* SNP rs 4580704 and MUFA intake for glucose plasma values and homeostatic model assessment: the protective effect of the minor allele on insulin sensitivity was only present when MUFA intake was >13.2 % of energy. We also found different effects across *CLOCK* 3111T > C genotypes for SFA intake (% of energy). The deleterious effect of C risk variant on waist circumference was only found with high SFA intakes (>11.8 %)⁽¹⁴⁾.

More recently, one study developed in 2014 by the present author group showed a novel *CRY* × diet interaction for insulin resistance in a Mediterranean population, that was also replicated in a North American population⁽³¹⁾. Findings from the meta-analyses indicated that an increase in carbohydrate intake was associated with an increase in HOMA-IR, in fasting insulin and a decrease in QUICKI, only among individuals homozygous for the minor C allele in *CRY1* polymorphism, rs2287161⁽³¹⁾. *CRY* (negative elements of clock) are implicated in the regulation of glucose metabolism. Studies in experimental animals have demonstrated that gluconeogenesis can be modulated by circadian changes in the hepatic expression of *Cry*⁽³²⁾. Therefore, factors which affect the expression of *CRY* could impair hepatic regulation of glucose homeostasis and thus increase risk for diabetes in human subjects⁽⁹⁾.

These results can help us design more effective dietary programmes: for those CC carriers at *CRY1* rs2287161, our advice would be focused on helping them reduce their carbohydrate intake so HOMA risk could be even lower than G carriers (see Fig. 1).

In another example, but this time for gene × drug interaction (i.e. exogenous melatonin), is related to the *melatonin receptor 1B* rs10830963 variant. Hence, *melatonin receptor 1B* rs10830963 risk variant G worsens the effect of exogenous melatonin on glucose tolerance, thus G carriers should avoid having food together with exogenous melatonin administration⁽³³⁾. These results could affect those subjects taking melatonin (i.e. for sleep problems), but also G carriers who usually have food (dinner) together with high levels of endogenous melatonin, as happens in natural late eaters or in shift workers. Further studies should answer these specific questions about timing of food intake, genetic risk and insulin resistance.

In Table 1, we include advice to prevent genetic and behavioural association/interactions to obesity that may be crucial for genetically informed personalised nutrition in obesity treatment.

All these examples and many others show us how our behaviours may interact with our genes and might decrease the deleterious effect of one specific risk variant. Consequently, to the question of whether or not we are predestinated nutrigenetics answers: no, we are not predestinated; even though we cannot change our genome, we can change our behaviours in order to improve our health.

Epigenomics says: we can change our genome with our behaviour

Presently, it is evident that genes are modulated by a set of regulators, which switch on or switch off altering the

Table 2. Effect of different behaviours in methylation levels of *CLOCK* CpG1, which shows the importance of ‘how we eat’

Evidences in epigenetic		
<i>CLOCK</i> CpG 1	Methylation levels	P value
Snacking frequency	12x	0.026
Eat fast	9x	0.08
Eat when bored	3x	0.008
Eat from large packages	19x	0.004

Milagro *et al.* Chronobiology International. 2012⁽¹⁾.

person’s phenotype. The field focused on the study of these genetic expression regulators is called epigenetics⁽³⁴⁾.

The main idea underlying this field is the following: epigenetics does not change our DNA; however, it decides how much or whether some genes are expressed in different cells of our body. These changes can occur as a result of three processes: (1) DNA methylation; (2) chromatin modifications; (3) post-transcriptional gene silencing mediated by RNA. The best characterised epigenetic mechanism is methylation, which consists of the addition of one methyl group to a cytosine of DNA when it stands next to guanine nucleotide (dinucleotide CpG, shorthand for ‘-C-phosphate-G-’) or cytosine and guanine separated by one only phosphate.

In this sense, it has been shown that circadian clock genes expressions can be regulated by epigenetic mechanisms⁽³⁵⁾. Moreover, methylation levels in clock genes have been associated with obesity and metabolic disturbances⁽¹⁾.

In 2012, the present group published a study, which demonstrated significant associations between the methylation levels of several CpG located in *CLOCK* with MetS, weight loss and obesity⁽¹⁾. This study, performed on sixty women, demonstrated that the methylation degree in the CpG sites of *CLOCK*, such as CpG 1, 5, 6 and 8, increased with obesity. More importantly, the ‘how’ we eat was related to methylation levels at *CLOCK*. We observed that those patients who tended to snack frequently had twelve times higher methylation levels in *CLOCK* CpG1, whereas those who tended to eat when bored or eat from large packages had nine to nineteen times higher methylation levels. These increases in methylation levels of blood mononuclear cells suggest a suppression of *CLOCK* expression, which has also been related to obesity. In Table 2, we have represented the effect of different behaviours in methylation levels of *CLOCK* CpG1, which shows the importance of ‘how we eat’.

More recently, research demonstrated that weight-loss nutritional intervention modifies the methylation pattern of *BMAL1*, *CLOCK* and *nuclear receptor subfamily 1, group D, member 1* in the whole blood. More importantly, these changes in methylation levels of *BMAL1* associated with a reduction in metabolic risk parameters, i.e. serum lipids⁽³⁶⁾. Interestingly, evening types presented more epigenetic modifications caused by the weight-loss intervention than morning types. Moreover, baseline methylation of *BMAL1* positively correlated with energy intake and carbohydrate intake, suggesting that interventions designed

NUTRIGENETICS

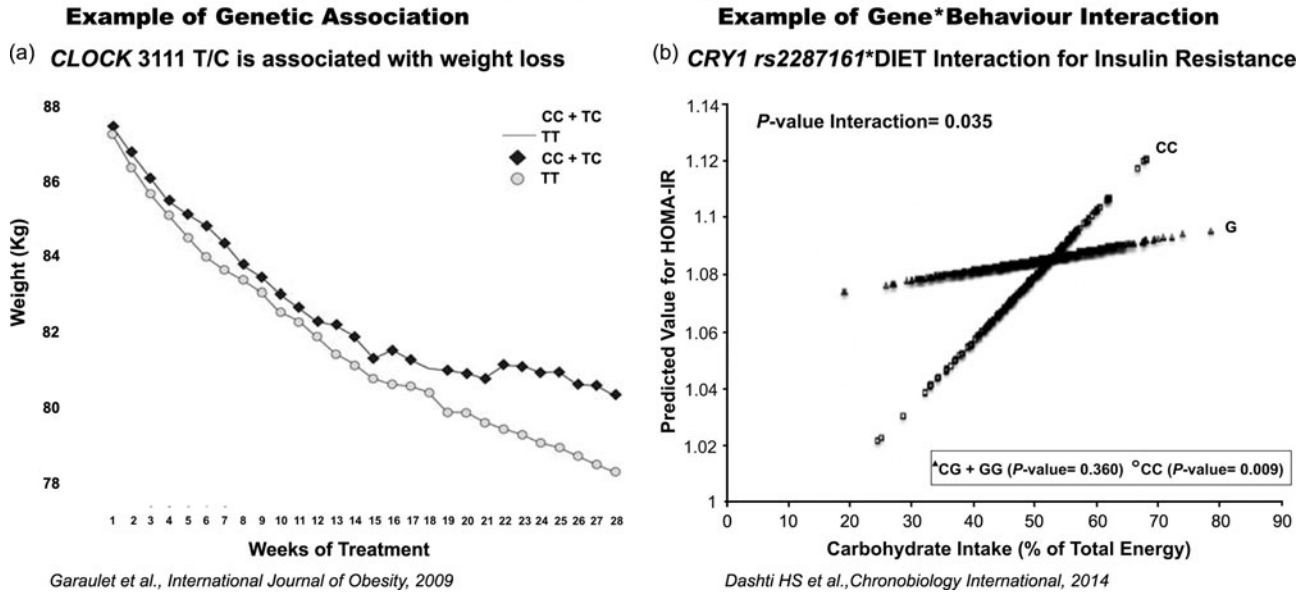


Fig. 2. Two typical examples of how behaviours may influence genetic variants. (a) Shows an example of genetic association between the genetic variant of *CLOCK* 3111T/C and weight loss, where the C carriers exhibit a greater difficulty in losing weight during 28 weeks treatment in a clinical nutrition centre than TT carriers. (b) Shows an example of gene × behaviour interaction for insulin resistance; it is remarkable that when subjects are CC carriers of *CRY1* rs2287161 and also eat high values of carbohydrates, their insulin resistance homeostatic model assessment (HOMA) is higher. However, among G carriers (GG + GC) values of HOMA are independent of carbohydrates intake and they remain constant.

to decrease total energy intake and carbohydrates may help decrease the vulnerability to CD and obesity, especially in evening type subjects (see Table 1).

What can we do? We can change what, how and when we eat

In this review, we have shown so far that we can interact with our genome, even change it, and improve our health through changes in how we eat, what we eat and other daily behaviour. One novel aspect to consider in dietary interventions may be when we eat: the timing of food intake. If we take into account that eating is an external synchroniser of our peripheral clock, and that an unusual eating time may cause a disruption of our circadian system^(37,38), the ‘when’ we eat may have a significant role in obesity treatment. In this line, a novel observational study performed in 2013 revealed that eating late may influence the success of weight-loss therapy, leading to a decrease of its effectiveness. This study was performed on 411 overweight and obese subjects who underwent a dietary weight-loss treatment: 199 subjects were early eaters (had their main meal of the day, lunch, before 15:00 hours) and 212 subjects were late eaters (had lunch after 15:00 hours). Late eaters lost significantly less weight than early eaters although having similar age, appetite hormones values, energy intake, sleep duration or macronutrients distribution. It is remarkable that late eaters were more evening types^(14,15). The conclusion of this study was that, if we could change our

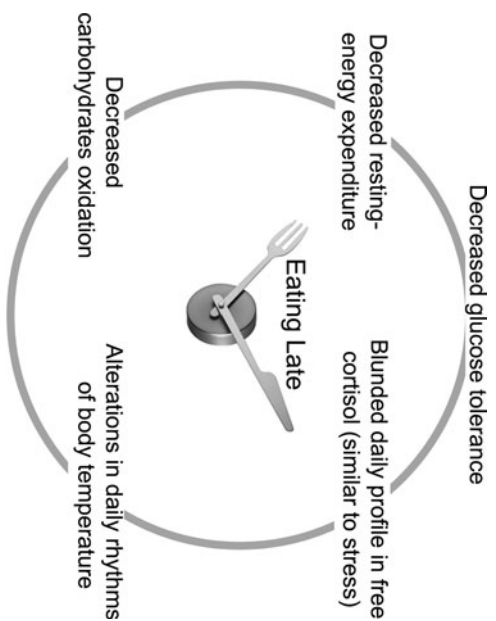
behaviour in Spain towards an earlier lunch, perhaps we could lose more weight during a dietary treatment.

More recently, another observational study developed in Barcelona showed that the timing of food intake might also influence the effectiveness of bariatric surgery in severely obese subjects. It is true that many subjects were good responders to the treatment (68 %) losing 80 % of their initial excess weight during the first year after surgery, and maintaining this weight loss during 6 years follow-up. However, about 11 % of the population is defined as ‘primarily weight loss poor responders’; these subjects lose only about 40 % of their initial excess weight during the first year after surgery. From a clinical point of view, it is important to detect this group of poor responders before the surgery, in order to make a decision about their further treatment. The results of this study demonstrated that the percentage of late eaters was significantly higher among the primarily poor weight-loss responders (about 70 %) than in good weight-loss responders (about 37 %). Moreover, poor weight-loss responders had lunch later than good weight-loss responders. Surprisingly, obesity-related variables, biochemical parameters, pre-surgical total energy expenditure, sleep duration, chronotype, energy intake and macronutrient distribution, were similar among groups⁽³⁾.

To discover why the timing of food intake could influence weight loss, we carried out a randomised study in which thirty-two lean and young women completed two protocols: one including assessments of resting energy expenditure (indirect calorimetry) and glucose

Table 3. Summarises the specific recommendations for the prevention of obesity and metabolic syndrome by improving the circadian system health, based on available scientific evidence proved in epidemiological, clinical and experimental animals (A); on interventional studies in human subjects or animal models (B); and on preliminary studies or low number of studies (C)

Recommendation	Evidence	Type of evidence
Sleep		
Do sleep during the night and be active during the day	Shift workers face potential health problems. Overall, those who work at night or rotating shifts seem to have a higher risk of insulin resistance, metabolic syndrome and heart disease	A
Sleep enough hours/d (7–8 h)	Insufficient sleep increases the risk for insulin resistance, type 2 diabetes, and obesity, suggesting that sleep restriction may impair peripheral metabolic pathways	A
Go to bed early and get up early	Late chronotypes have been previously related to obesity, less healthy and more irregular lifestyles	A
Sleep siestas in the middle of the day of short durations (20 min)	Napping for 90 min, too late in the afternoon, can impact on night time sleep towards a reduced sleep efficiency and earlier wake times	B
Light		
Avoid intense light exposition during night time, and sleep in total darkness when possible	Light is an external synchroniser of circadian rhythms	A
Meal timing		
Eat the main meal of the day before 15:00 hours	Eating late is associated with several metabolic alterations	A
Eat dinner at least 2 h and a half before going to bed	The coincidence of melatonin and food intake (glucose) is associated with impaired glucose tolerance	B
Avoid eating at night hours	Increasing scores on the night eating questionnaire (NEQ) correlated positively with increasing BMI and blood pressure in women. NEQ scores correlated positively with waist circumference and triacylglycerol levels in men	C
Timing in physical activity		
Avoid performing physical activity during the 2 or 3 h previous to habitual sleep time	Evening physical activity alters wrist temperature circadian rhythmicity	C



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Fig. 3. Represents how eating late affects the metabolic characteristics of lean women; showing that, after 1 week of late eating, their metabolic pattern was more similar to the metabolic pattern of obese women.

tolerance, and the other including circadian-related measurements, such as profiles in salivary cortisol and WT (Fig. 2)⁽²⁾. Participants received standardised meals during both meal interventions weeks and were studied under two timings: early eating (lunch at 13:00 hours) and late eating (lunch 16:30 hours). The results of this study showed that these lean women, after 1 week of eating late, suffered from metabolic alterations that usually characterise obese women, such as decreased glucose tolerance, decreased resting energy expenditure and decreased carbohydrate oxidation. Moreover, late eaters had a flattened pattern of daily cortisol and alterations in daily rhythms of body temperature similar to those that characterise obese women as previously described⁽³⁹⁾.

In Table 3, we summarise the specific recommendations for the prevention of obesity and MeS by improving the circadian system health, based on available scientific evidence proved in epidemiological, clinical and experimental animals (A); on interventional studies in human subjects or in animal models (B); and on preliminary studies or low number of studies (C).

These recommendations can be very useful for the general population and also in the clinical practice to prevent or treat obesity alterations.

Summary

In Fig. 3, we represent a summary of the current revision. As it is shown, several impairments in the central clock are related directly with obesity. In experimental animals, these impairments are due to mutations; however in human subjects, mutations are rare and these impairments are caused by human genetic variants. Thus, these human genetic variants may be associated directly with obesity or through different behaviours. Moreover, these genetic variants may interact with different

behaviours to influence obesity and weight loss. We can consider several solutions: (a) changing our behaviours as these changes are directly related to a decrease in obesity and an increase in weight loss; or (b) through genetics by changes in our behaviour that may interact with the SNP to produce a decrease in obesity and weight loss.

In conclusion, herein, we propose modifying what, how and when we eat as an effective tool to decrease our genetic risk, and as a consequence to diminish CD and to decrease obesity. The research addressed in this revision presents a novel and very promising area in obesity prevention and treatment.

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Conflicts of Interest

None.

Authorship

All authors contributed equally to the preparation of this paper.

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