UNIVERSITAT DE BARCELONA

The impact of nutrients on clock genes and metabolism: their role for the prevention and treatment of metabolic diseases

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Memoria presentada por Ana Lucía Castillo Figueroa para optar al título de doctor por la Universidad de Barcelona

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Abbreviations

3T3-L1	3T3 preadipose murine cell line
ACO	Acyl coenzime A oxidase
ADP	Adenosine diphosphate
AGEs	Advanced Glycation End products
AGRP	Agouti-related protein
AMPK	Adenosine monophosphate-activates protein kinase
ARNT	Aryl hydrocarbon receptor nuclear translocator
ATP	Adenosine triphosphate
BMAL1	Brain and muscle Arnt -like protein-1
BMI	Body mass index
C/EBP A	CCAAT/enhancer binding protein alpha
C/EBP B	CCAAT/enhancer binding protein beta
CCGs	Clock Controlled Genes
CDO	Cysteine dioxygenase
CLOCK	Circadian locomotor output cycles kaput
CNS	Central nervous system
CRY	Cryptochrome
CRY ½$^{-/-}$	Cry1 and Cry2 global mutant mice
CSAD	Cysteine sulphinic acid decarboxylase
CTP1	Carnitine palmitoyltransferase I
CVD	Cardiovascular disease
CYP7 α 1	Cholesterol 7 α -hydroxylase
DBP	D-Box Binding PAR
DD	12:12 h dark/dark, constant darkness
ELISA	Enzyme-linked immunosorbent assay□
FEO	Food entrainable oscillator
FAA	Food anticipatory activity
FFA	Free fatty acids
G6PASE	Glucose-6-phosphatase
GABA	Gamma-Aminobutyric acid
GCK	Glucokinase
GLUT 2	Glucose transporter 2
GLUT 4	Glucose transporter 4

GSIS	Glucose stimulated insulin secretion
HSL	Hormone sensitive lipase
H_2S	Hydrogen sulphide
IL-6	Interleukin 6
INS2	Insulin
i.p	intraperitoneal
K_{ATP}	ATP-sensitive potassium channel
KO	Knock out
LD	12:12-h light/dark
LL	12:12-h light/light
MCP1	Monocyte chemotactic protein-1
mRNA	Messenger RNA
NAD	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NES	Night-eating syndrome
NFR2	Nuclear factor (erythroid-derived 2)-like 2
NOC	Nocturnin
NR	Nuclear hormone receptor
NTCP	Sodium/Taurocholate Co-transporting Polypeptide
ob/ob mice	Leptin-deficient mice
PCR	Polymerase chain reaction
PDX1	Pancreatic And Duodenal Homeobox 1
PER 1	Period 1
PER 2	Period 2
PGC1 *α*	Peroxisome proliferative activated receptor, gamma, coactivator 1
POMC	Propiomelanocortin
PP cells	Pancreatic-polypeptide cells
PPAR*γ*	Peroxisome proliferator activated receptor gamma,
PVN	Paraventricular hypothalamic nucleus
RBP4	Ribosomal binding protein 4
RDA	Recommended Dietary Allowances
REV-ERB *ß*	NR1D2 - Nuclear receptor subfamily 1, group D, member 2
REV-ERB *α*	NR1D1 - Nuclear receptor subfamily 1, group D, member 1
RNA	Ribonucleic acid
ROR	Retinoid-related orphan nuclear receptor

ROS	Reactive oxygen species
Rpm	Revolutions per minute
Rt	Room temperature
SCD1	Stearoyl-CoA desaturase 1
SCN	Suprachiasmatic nucleus
SIRT1	Sirtuin 1
SNPs	Single Nucleotide Polymorphism
T2DM	Type 2 diabetes mellitus
Tau	Taurine
TAUT	Taurine transporter
TG	Triglycerides
TGF-ß	Transforming growth factor beta
TNF A	Tumor necrosis α
WAT	White adipose tissue
ZN^{2+}	Zinc cation

Units

mM	mili molar
mmol	mili mole
g	gram
Kg	kilogram
mg	milligram
mg/d	milligram per deciliter
h	hour
ng/ml	nanogram per mililiter

PRESENTATION

The importance of the circadian rhythm in regulating human food intake behavior and metabolism has long been recognised, the past 2 decades have had a wide increase in understanding how the generation and organization of circadian rhythms control fundamental physiological functions in the organism. However changes disturbing these rhythms can profoundly influence human health and placing additional stress on our bodies that ultimately affects our diet and our health. Due to the known connection to metabolism, metabolic disorders such as obesity and diabetes are linked to the circadian clock. The role of nutrition on our circadian clocks is emerging; diet plays an important role in this field and has also been proposed as a powerful re-entrainment mechanism to circadian alteration as well. Despite the amount of translational work required, there are already encouraging signs that the application of basic models of circadian biology can have a major effect on human obesity.

Identifying nutritional strategies to alleviate the obesity pandemic are of great interest. An important area of future interest for the field, regarding functional nutrients, we focused such is the case of taurine essential role in the body, supplementing with taurine can provide numerous health benefits. Is clearly important in view of effective preventive measures for public and individual patient treatment strategies. We need to focus in this type of compounds as an applicable tool for humans and as an important factor included in diet or as supplements to current approaches, representing part of the interface linking different nutrients with the circadian clock and metabolic diseases.

1. INTRODUCTION

1.1 Physiology of circadian rhythms and the biological clock.

Circadian rhythms were first discovered in plants nearly three centuries ago, and since then have been observed in nearly all kingdoms of life. [1] Biological rhythms exists in almost all organisms extending from bacteria, plants, to evolved mammals and are sustained by an endogenous clock that optimally generates circadian rhythms of almost 24-h. Circadian means (from the Latin "*circa diem*" meaning *about a day*) and it refers to a cycle of approximate 24h. The daily rotation of the earth leads to different changes in the planet, and those include the movement from day and night. For mammals this has evolved to adjust the organism according to the light and dark cycles[2]. Circadian rhythms have two main properties; they are entrained normally to the solar cycle and can maintain its endogenous properties in the absence of external signal. After exclusion of all environmental time cues, for example, under constant darkness, circadian rhythmicity persists but with a free-running rhythm slightly deviating from 24 hours with a period close to 25h showing that the existence of a biological clock makes a rhythm viable even in the absence of environmental cycles [3].

Such endogenous rhythms are driven by a group of genes called clock genes that conform the circadian system [4]. This system ensures homeostasis in an extended range of behavioural and physiological processes that take place at the right time of the day. In mammals many physiological processes like sleep-wake cycles, locomotor activity, body temperature, hormone secretion, blood pressure, heart rate and metabolism are under control of circadian clocks, this ability to sustain an internal time keeping mechanism in order to optimize energy utilization, health, longevity and survival of the organism has been a target of research from more than three decades that have supplied new advantage and knowledge about how the biological clock affects directly human physiology [5,6].

1.1.1 The molecular clock machinery

In mammals circadian clocks genes exists throughout the body, in individual cells and tissues, and are regulated by a molecular feedback loop formed by a positive and negative arm that is highly conserved between species. The core clock is composed by the transcription factors clock circadian locomotor output cycles kaput (CLOCK) and brain and muscle Arnt-like protein-1 (BMAL1), that bind to E-box sequences in their target gene promoters, Period (*Per*) [7] and Cryptochrome *(Cry*) [8] inducing their expression. Once in the cytoplasm PER and CRY proteins, form a complex and translocates into the nucleus to inhibit the transcription of *Clock* and *Bmal1* repressing their own gene expression. The autoregulatory transcription–translation loop composed by CLOCK:BMAL1 and PER:CRY generates rhythms of gene expression of approximately 24-h [9]. In addition, the cyclic activation of Clock /Bmal1 modulates the circadian expression of the nuclear receptor reverse erythroblastosis virus a (REV-ERB α). *Bmal1* expression is inhibited by the transcription factor Rev-erb α and activated by retinoic acid receptor-related orphan receptor a (ROR α) (**Figure 1).**

This transcriptional feedback loop is regulated by complex mechanisms as posttranslational modification of circadian proteins such as phosphorylation that affect stabilization, degradation, and subcellular localization of clock proteins. These mechanisms are essential for clock protein turnover and for entrainment and synchronization with the environment [10].

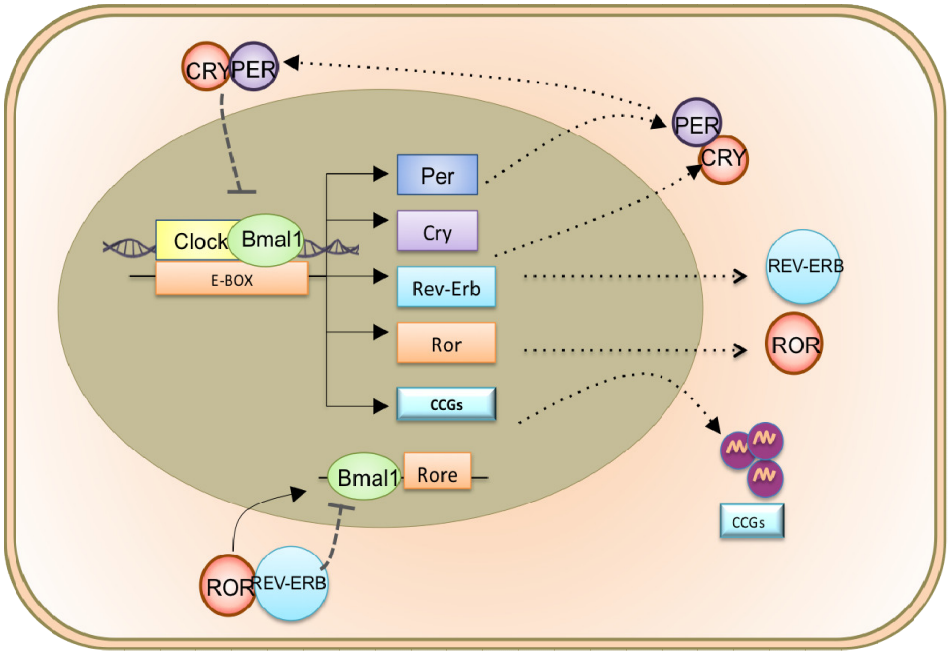


Figure 1. Feedback loop in the core circadian clock.
The positive arm, Clock and Bmal1, drives the transcriptional expression of the negative arm, Per and Cry. PERS and CRYs undergo nuclear translocation and inhibit Clock:Bmal1, resulting in decreased transcription of their own genes. Clock:Bmal1 heterodimer also induces the transcription of *Rev-erb* and *Ror*. Diverse clock controlled genes (CCGs) involved in metabolic rhythms throughout the organism are regulated by the circadian clock. (Adapted from Vieira *et al* Trends Mol Med 2014).

1.1.2 The master clock

Molecular circadian clock genes are present in most mammalian cell types. The master clock is located in the suprachiasmatic nucleus (SCN) of the brain anterior hypothalamus. The SCN clock functions as the pacemaker, composed of around 20.000 neurons that integrate multiple single-cell oscillators to generate circadian rhythms in behavior and physiology [11]. SCN lesion and transplantation experiments have demonstrated that SCN is both necessary and sufficient for sustained circadian rhythms under most experimental conditions. The first role was showed by lesion studies in the SCN of rodents in which circadian rhythms of locomotor activity and food consumption were abolished. Transplantation of SCN grafts into SCN lesioned animals

recovers circadian rhythmicity, and feeding behavior by the SCN donor [12,13]. Several studies indicate that failure in the SCN alters systemic energy homeostasis. For instance animals with specific SCN injury display insulin resistance, increase in body weight, and alter daily rhythm of activity and food intake [14]. Additionally, the SCN is responsible for a 24-h rhythm in plasma glucose concentrations showing a direct control of glucose production and uptake, resulting from cross-talk of the SCN and paraventricular nucleus (PVN) [15]. The SCN tissue slices maintained in vitro are also capable of maintaining self-sustained oscillations *in vitro* for several weeks displaying circadian rhythms of neural activity that persist for several days [16,17]. In this way, the circadian system ensures that organisms can synchronize with changes in the environment, matching internal physiology with external cycles. Several stimuli are capable of entraining the circadian system. Among different time cues that phase shift circadian clocks called *zeitgebers* from the german *Zeit* (time) and *geber* (giver), which are environmental signals such as light, food, temperature, exercise, and drugs. Light is the major environmental stimulus responsible for the entrainment of the SCN to daily changes. This input is perceived by a particular type of retinal ganglion cell containing the photopigment melanopsin, responding intrinsically to photic stimulation, then transmitted to the SCN through the retinohypothalamic tract (RHT) [18]. The SCN signalling is passed on via neural projections to different hypothalamic areas which express daily oscillations as well [19]; then progressive signals are sent to peripheral clocks via neuroendocrine and autonomic nervous system [20,21]. The central clock has anatomic connections with several regions of the central nervous system (CNS) involved in the control of appetite, energy expenditure and behavioral activity. From the PVN where the SCN directly target and is one of the major place which projects the information from the SCN to the rest of the body. The PVN is the hypothalamic center for autonomic control, which includes neural connections release of neuropeptides and

SCN driven rhythmic hormone regulation [22,23].

1.1.3 Peripheral clocks

It has been demonstrated that not only the SCN but also most tissues are able to sustain rhythms of circadian gene expression. While the master clock in the SCN is mainly synchronized by light, food is an important synchronizer to peripheral clocks. Tissue explants from different peripheral tissues of rodents shows persistent rhythms indicating that the molecular clock work acts autonomously within individual cells [24, 25]. Circadian clocks genes are functional in most body cells; several studies in animals have demonstrated clock oscillations in liver, adipose tissue, pancreas, stomach, intestine, muscle, lung, kidneys [26,27]. An important characteristic of mammalian peripheral clocks is that their oscillations dampen very rapidly *in vitro*, similar to what has been observed in Drosophila and Zebrafish peripheral tissues [28,29]. In peripheral organs a large number of key physiological functions are subject to daily oscillations including glucose production, fat storage, and hormone secretion [30,31]. Some of the important questions are beginning to answer in relation to these pathways, depending on various signals controlled by the SCN and environmental factors that participate in the synchronization of clocks in peripheral cell types and organs.

It is possible that peripheral clocks are altered before changes in metabolism. When leptin is lacking, expression *of Clock, Bmal1, Cry1, Per1, Per2*, and *Dbp* in adipose tissue and liver were altered in deficient leptin mice (ob/ob), independently of metabolic alterations. Furthermore, repeated treatment with leptin at the light–dark cycles partially rescues the hepatic oscillations of clock and clock-controlled genes in ob/ob mice. Suggesting that altered clockwork can play a role in obesity in addition to the specific deficiency of leptin [32]. But further studies are needed to understand this feedback

regarding alteration of peripheral clock genes as a cause or consequence of metabolic diseases.

1.1.4 The liver clock

The liver is a central metabolic organ involved in glucose, amino acid and lipid homeostasis. Clock genes play a significant role in the regulation of hepatic function. Metabolomic studies have shown that a wide diversity of hepatic metabolites oscillate in a circadian manner [33]. Liver clock gene expression also modulates both bile acid and apolipoproteins biosynthesis and has a clear diurnal variation with the highest activity during the day [34].

Metabolic alteration in clock genes mutant shows the relevance in liver metabolism. It has been studied that liver-specific *Bmal1* mutant mice reduces oscillations of the retinol binding protein 4 (RBP4) in liver mice. RBP4 is suggested to be positively associated with insulin resistance and acts as a hepatokine in the regulation of glucose metabolism via clock genes- regulation [35]. In addition, liver specific deletion of Bmal1 regulates the plasma levels of low-density lipoproteins (VLDL) and lipid-associated genes in the liver [36]. The circadian regulation of liver metabolism is well demonstrated by studies on the rate-limiting gluconeogenic enzyme, phosphoenolpyruvate carboxylase (PEPCK). The PEPCK activity is diurnal in mouse liver, contributing to the diurnal rhythm of hepatic glucose production [37]. For example *Clock* mutant mice ($Clock^{mut}$) may be involved in direct regulation of PEPCK in liver. These animals also display an altered diurnal variation in triglycerides (TG) [38]. Beside *Bmal1* and *Clock*, interestingly REV-ERBα also regulates normal hepatic physiology. Whole-body knock out (KO) mice of *Rev-erbα* results in hepatic steatosis with a large increase in triglyceride and impaired rhythmic expression of genes involved in bile synthesis and hepatic lipid metabolism. REV-ERB*α* regulates the transcription of sterol element

binding protein 1c (SREBP1c), which is expressed in a diurnal manner in the liver. In addition plasma and hepatic levels of cholesterol and lipoproteins are altered in these mutant mice [39–41].

Other regulators are Period genes, which have been demonstrated to be involved in bile acid metabolism. *Per1/Per2* double knockout mice (*Per-dKO*) showed accumulation of bile acids in the liver that lead to hepatic cholestasis. Diverse key genes of bile acid pathways, including cholesterol-7a hydroxylase (*Cyp7a1*) and sodium bile acid transporter (*Ntcp*), show altered circadian oscillations in these mice [42,43]. The circadian clock may also be involved in the hepatic glycogen metabolism. Glycogen content in the liver is important for glucose homeostasis and exhibits a robust peak at the end of the active phase in mammals [64]. *Per2* mutant mice showed a loss of rhythmic glycogen accumulation in the liver of these mice [44]. The liver clock responds differently to maintain metabolic homeostasis, however liver clock has not been well investigated in the context of therapeutical targets and clinical studies. Thus, future research is aimed to understand the liver circadian clock regarding hepatic physiology and disease.

1.1.5. The Adipose Tissue Clock

Adipose tissue has been recognized as an important metabolic organ. It is involved in the regulation of appetite, energy expenditure, insulin sensitivity, endocrine and reproductive systems, inflammation and immunity. It is heterogeneous in composition and contains, besides mature adipocytes, also immature adipocytes (preadipocytes), endothelial cells, fibroblasts, macrophages and other immune cells. [45]. Adipose tissue synthetizes and releases hormones that are collectively referred to as adipokines which include leptin, adiponectin, resistin and visfatin that participates in the regulation of

whole body homeostasis through endocrine, autocrine and paracrine activities [46]. In addition to hormones, diverse products of adipose tissue have been characterized, including cytokines and growth factors such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), monocyte chemo attractant protein 1 (MCP1), and others. The accumulation of fat mass during the development of obesity is characterized by hyperplasia and hypertrophy of adipocytes and is associated with a state of chronic, low-grade inflammation through different mechanisms [47]. This inflammation is characterized by increased infiltration of immune cells and production of proinflammatory cytokines, which are key mediators of the inflammatory process [48]. In addition to increased number of immune cells in adipose tissue, lipotoxicity from high concentrations of free fatty acids (FFA), ectopic fat deposition in the liver and fat depots, hypoxia, and apoptosis may promote obesity-linked metabolic disorders such as insulin resistance, metabolic syndrome and atherosclerosis[49 50]. It is established that expression of the circadian clock in adipose tissue could influence adipose tissue function. Normal variations in body weight associated with periodically changes in day have been observed in different mammals, suggesting a role for the circadian clock mechanism in body weight control and adiposity. In epididymal fat, inguinal fat and brown adipose tissue, clock genes show 24h rhythms [51]. In addition excess adipose tissue and altered body fat distribution are an important risk factor for obesity-associated diseases. The role of clock genes may vary among different types of adipose tissue. Analysis of subcutaneous fat from obese humans shows no abnormalities in the rhythmic transcription of clock genes [52]. *Ex vivo* analysis of human visceral adipose tissue in lean and obese people showed that expression of peripheral clock genes are altered in isolated adipocytes. CLOCK and BMAL1 were upregulated in adipocytes from obese patients at different time points. Also CRY2 and REV-ERB α had increased expression levels over the 24-hour time. A positive correlation was

observed for REV- ERBα gene expression with BMI and waist circumference, ROR α expression was correlated with HDL levels and CLOCK expression was correlated with LDL levels in obese humans [53]. Therefore, evidence from both experimental and human studies suggests that expression of the circadian clock in adipose tissue could influence adipogenesis and distribution of fat depots [54,52]. The importance of circadian mechanisms in driving circadian adipokine secretion has been shown in rodent models, in which adipokine secretion are dependent on a functional SCN and continues to fluctuate in the absence of light [55]. A recent study linking circadian clock, obesity, and leptin resistance, focus on circadian clock disruption with chronic jet lag in *Per* and *Cry* mutant mice. Under normal diurnal rhythms or chronic jet lag, the authors show that energy balance and body weight are disturbed in different ways, depending on the KO mice. They found a direct circadian control of leptin expression in adipocytes from white adipose tissue through BMAL1/CLOCK modulated by CCAAT/enhancer-binding protein alpha (C/EBPα) which mediates leptin transcription. They also show that chronic jet lag is sufficient to disrupt adipose clock and also induce central leptin resistance in wild-type mice [56]. Suggesting that leptin feedback loop is a key mechanism for the adipose tissue clock to control long-term energy balance.

In adipose tissue, clock genes controls the expression of genes involved in lipid metabolism. The clock gene *Bmal1* is essential regulator of adipogenesis and lipid metabolism. *Bmal1* deletion in matured adipocytes induces several factors involved in lipogenesis [57]. In addition embryonic fibroblast cells deficient in *Bmal1* fail to differenciate into adipocytes. Also *Bmal1* KO mice increases circulating fatty acids and ectopic fat formation [58]. Apart from *Bmal 1* another clock genes were shown to regulate adipocyte function, the nuclear receptor REV-ERB*α* participates in the regulation of diverse metabolic pathways, including adipocyte differentiation. Recently it was found that REV-ERBα modulates the development of brown adipose tissue, promoting de

novo brown adipocyte formation by repressing genes of the transforming growth factor beta (TGF-β) pathway which is a inhibitory pathway in brown fat development [59]. In addition *Rev-erbα* mutant mice are more susceptible to dietary obesity and weight gain, as well as an increase of retroperitoneal and perigonadal white adipose tissue weight in response to metabolic stress.[60]

In parallel to direct regulation by clock genes, rhythmic expression of a majority of nuclear receptors in adipose tissue could contribute to the regulation of adipocyte function. PPARγ is a nuclear receptor that is highly expressed in adipose tissues, controlling adipocyte differentiation and insulin sensitivity [61,62]. In primary rat adipocytes as well as in vitro cell line of differentiated 3T3- L1 adipocytes, *Pparγ* activation drives *Rev-erbα* expression by inducing Rev-erbα promoter activity, binding to the Rev-DR2 site, identifying *Rev-Erbα* as a target gene of PPAR in adipose tissue. *Rev-Erbα* also induces PPAR expression and the expression of the PPAR target genes, adipocyte protein 2 (aP2), and CCAAT-enhancer-binding proteins (C/EBP), tin this manner having a reciprocal autoregulation [63]. Another clock gene involved in adipose function is *Clock*; specifically the Clock Δ19 strain in mice has been studied to elucidate the functional role in peripheral tissues. Clock Δ19 mutant mice show an increase epididymal fat weight, adipocyte size and higher body weight gain. These mice also show blunted release of FFA, a decrease and altered pattern in glycerol serum concentrations. The expression of genes involved in lipid metabolism as lipolytic enzymes like adipose triglyceride lipase (*Atgl*) and hormone-sensitive lipase (*Hsl*) also present a reduced activity and hence general down regulation of basal lipolysis. In this manner clock genes regulation provide a rhythmic deliver of FFAs and glycerol from adipocytes [64]. Other studies involving Per genes, demonstrated that *Per* 1/2 global mutant mice shows a higher total body fat composition whereas Per2 mutants enhanced adipocyte differentiation and lack of *Per2* is altered normal lipid metabolism

in white adipose tissue. In addition PER2 controls the proadipogenic activity of PPARγ [56,65].

Another factor affecting fat absorption, mobilization, lipogenesis, and energy homeostasis is nocturnin (*Noc),* a clock-controlled gene (CCG) regulated by CLOCK/BMAL1, which encodes a circadian deadenylase involved in post-transcriptional mRNA regulation [66]. Nocturnin is expressed at high levels during the night in a number of tissues especially liver and adipose tissue in mammals and has been implicated to regulate lipid metabolism and to control preadipocyte differentiation [67]. Recent evidence from mice lacking nocturnin, suggested a mechanistic link between nocturnin and Pparγ in adipogenesis, also these animals display resistance to diet-induced obesity [68]. Increasing evidence supports a pivotal role for nocturnin in lipid metabolism, but many questions remain regarding the precise mechanisms and its role in other tissues. Thus, the clock machinery seems to have significant metabolic implications in fat depots. Future studies of the circadian clock in adipose tissues will help to delineate more precisely and provide novel therapeutic approaches.

1.1.3.1 Circadian hormone regulation

A major output pathway of the circadian clock is the endocrine system, which allows for a systemic coordination of various physiological functions. It has been shown that the circulating levels of a number of hormones vary over the 24-h cycle. The endocrine system and its role in the secretion of hormones have a feedback signaling on central and peripheral clocks. A functional advantage of circadian outputs mediated by hormonal rhythms is that they can exert a broad influence circadian clock gene expression in a number or peripheral tissues like liver, adipose tissue, stomach, pancreas.

Glucocorticoids

Glucocorticoids, a group of steroid hormones synthesized in the adrenal cortex are fundamental regulators of energy metabolism as well as stress responses. The circadian clock transcriptionally regulates clock genes are expressed rhythmically in the adrenal gland, and entire pathways characteristic for the adrenal gland, like steroid metabolism or catecholamine production [69]. Glucocorticoids are driven by the SCN and have been suggested as crucial humoral signals for transmitting daily rhythms to the body. Cortisol and corticosterone concentration display a diurnal variation. Cortisol is secreted in a circadian rhythm with high levels in the early morning and low levels in the evening and night. Plasma corticosterone levels have a strong diurnal rhythm, with a peak near habitual wake time, strongly driven by the master circadian clock [70,71].

On the other hand, pathologically high levels of cortisol are associated with abdominal obesity, insulin resistance and dyslipidemia in Cushing's disease [72, 4]. For example It is well known that Cushing's syndrome is associated with a disturbed circadian rhythm; patients with Cushing's syndrome show increased basal cortisol levels as well as an altered daily rhythm [73]. In addition these hormones are influenced by light, so changes in light and dark cycles could result in disruption of the circadian rhythm of cortisol secretion. Furthermore, molecular interactions between the glucocorticoid receptor (GR) and clock components have been studied. The glucocorticoid nuclear hormone receptor is expressed in virtually all peripheral cell types, except in SCN neurons The application of dexamethasone, an agonist of the glucocorticoid receptor, is a potent synchronizer for clock genes as demonstrated on rat fibroblasts and *in vivo* treatment in rats but does not affect SCN rhythms [74]. Among the genes found to be regulated by glucocorticoids in a pulsatile manner is *Per1*. In vivo, an injection of GR agonist on rats induces a phase shift of *Per1* in the liver [74]. In fact Per1 mutant mice exhibited

increased levels of corticosterone, which suggests that corticosterone rhythm might be dependent of a functional clock [75]. As well It has been shown that CRY1 and CRY 2 interact with glucocorticoid receptor in mouse liver by repressing it [76]. Thus it seems to be an interaction over different scales. While recent work has made increased progress to understand the molecular mechanisms and cross talk between glucocorticoids and the circadian clock, many open questions remain.

Melatonin

Melatonin has been linked to peripheral clock regulation. Melatonin is a hormone secreted during the night by the pineal gland, its daily rhythm is one of the major efferent endocrine outputs of the SCN clock and the most studied physiological effects are attributed to the sleep-wake cycle regulation. Beyond its effects in sleep also may be act as the main pineal secretory hormone regulating glucose metabolism and as an internal synchronizer [77]. There is evidence that the absence of rhythmic release of melatonin in blood circulation leads to alterations associated with energy metabolism. In rats subjected to pinealectomy, the absence of melatonin led to decrease in spontaneous locomotor activity and a shortening of the free-running period of activity, also these animals decreased amplitude of rhythmicity of mRNA levels of *Per1, 2* and *Bmal1* in liver and intestine [78]. Moreover, nocturnal release of melatonin can provide temporal cues to target tissues expressing melatonin receptors. In KO mice for both melatonin receptors MT1 and MT2, the daily profiles of clock gene expression of *Per 1, Dbp* and *Rev-erb α* are altered in the liver and pancreas and an up-regulation of insulin secretion was detected in isolated islets of MT1 and MT2 KO mice [79]. Moreover, melatonin cues influence the phase of other hormonal rhythms; in pinealectomized hamster, leptin secretion is no longer rhythmic, Therefore suggesting that circulating melatonin at night drives the daily rhythmicity of plasma leptin, participates in the phase control of cortisol rhythm and modulates glucose homeostasis [80,81]. In this manner

melatonin appears to be an important hormone that is to likely respond to multiple signals in a tissue specific manner and an effective endocrine signal for peripheral clocks.

Ghrelin

An important hormone associated with hunger is ghrelin, an orexigenic peptide secreted by the oxyntic cells of the stomach which possess a functional clock [82,83]. Plasma levels of ghrelin vary according to the feeding cycle increasing during the resting period in anticipation of food intake and decrease once the animal has been fed in nocturnal rodents [84].

Ghrelin elicits feeding primarily through activation of neurons in the arcuate nucleus (ARC), resulting in increased release of neuropeptide Y (NPY) and Agouti-related protein (AGRP) in the paraventricular nucleus of the hypothalamus (PVN) [85]. Ghrelin from the digestive system, may reach the CNS to act as a potential feedback signal for the SCN [86]. In respect to the SCN, in vitro cultured SCN slices can directly respond to ghrelin, being capable to reset the master clock. Furthermore, when injected *in vivo* in transgenic mice carrying PER:2 luciferase fusion protein as a reporter, it produces phase shifts of locomotor activity in fasted conditions, but not in fed animals [87]. Despite its role as a peripheral feedback signal to the central circadian system, little is still known about how the circadian system affects ghrelin secretion and sensitivity.

Leptin

Among the known endocrine products produced by adipocytes, the strongest evidence exists for leptin to have a critical role in regulating energy balance via its actions on

food intake and energy expenditure. Secretion of leptin, by white adipose tissue, has a broad impact on metabolism through its action on peripheral organs or in the CNS, and more specifically in the hypothalamus where leptin acts as a strong appetite inhibitor [88]. Evidence suggests the possible role of rhythmic leptin as an internal time-giver closely linked to peripheral clocks. Leptin levels in the blood exhibit oscillatory pattern as well. In rodents, levels of circulating leptin are high during the active period, which peaks in early night in nocturnal and diurnal species [89]. Circadian variations of circulating leptin have been also reported in humans, but unlike rodents, the peak of leptin secretion occurs at night during the fasting/sleep period, favoring a decreased appetite. Meal timing also impacts the daily rhythm of leptin secretion in humans [90], which is closely associated with feeding and insulin release in both humans and rodents [91]. In most obese individuals, leptin fails to suppress appetite despite increased blood leptin concentrations; a condition that has been termed, in reference to insulin resistance in type 2 diabetes, as leptin resistance, although the mechanism of this lack of leptin action has still to be determined [92].

The daily rhythm of leptin release is under the control of the SCN clock via its autonomic input to the adipose tissue. Indeed, the leptin rhythm, in rats, persists in adrenalectomized animals but disappears after an SCN lesion [55]. Leptin is also implicated in the regulation of other circadian physiological functions, such as glucose metabolism. Recently it was found that mice lacking functional leptin receptor (db/db) and deficient leptin mice (ob/ob) disrupted daily pattern of glucose. Animals treated with exogenous leptin at night could modulate blood glucose rhythm, which advanced the glucose rhythm, while the same injections applied during the light period cause arrhythmicity of glycemia [93]. This suggests that rhythmic leptin can be a determinant of daily variations of blood glucose. Therefore leptin is a crucial hormone involved in

many pathways, having a great potential for clinical use as potential therapeutics for obesity that could be critical for the entrainment and restoration of the circadian clock.

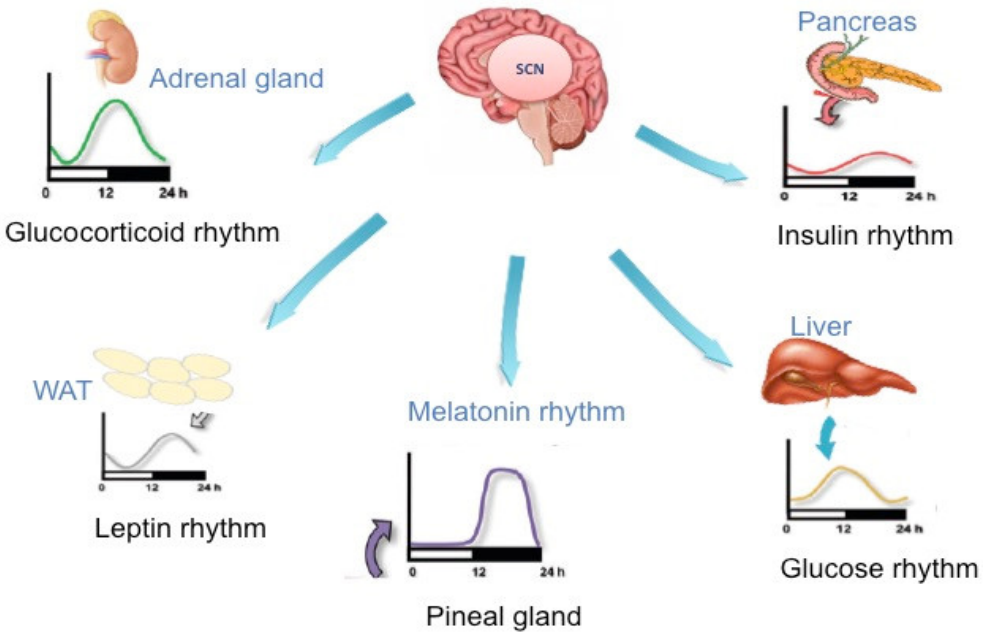


Figure 2. Endocrine regulation and circadian clocks

Hormonal rhythms are important components of the circadian timing system. Melatonin always secreted at night participates in internal synchronization by its time-giving role on the brain, including the SCN and peripheral organs. Corticosterone, secreted at night in nocturnal rodents also participates in internal synchronization by its timing effects on various cerebral and peripheral structures. Leptin, secreted from adipocytes at night in nocturnal rodents influences the phase of glucose rhythm and inhibits behavioural anticipation of feeding time. Feeding-induced rise in plasma insulin triggers clock gene expression in peripheral organs, such as the liver. Hunger-induced rise in plasma ghrelin stimulates behavioural anticipation of meal time. (Challet *et al* Diabetes Obes Metab 2015)

1.2. Nutrient sensing, metabolism, and the circadian clock

Large research in rodent and human studies shows that circadian clock system is highly influenced by nutrient intake. As well circadian control of digestion and metabolism may have important implications for many aspects of feeding behavior. Recently, it has been emerged the concept chrono-nutrition, to refer the relationship between food and the circadian clock according to food administration in coordination with the body's daily rhythms. Chrono-nutrition comprise two points; the timing of food intake or contribution of nutrients for health maintenance, and contribution of nutrients that induce changes in the circadian clock [94]. Chrono-nutrition involves involved different eating patterns that have been described recently in the literature.

1.2.1 Timing of food intake

Many aspects of diet and lifestyle influence metabolic status and disease development during life; emerging findings suggest that the influence of the frequency and timing of meals could be extensive in terms of beneficial effects in health. Interestingly, the synchronizing effect of foods may vary depending on the time of the meal. In humans it has been demonstrated that people with night eating syndrome (NES) often present overweight or obesity and inability to lose weight. NES comprise an excessive food intake at night and problems in sleep pattern [95,96]. Timing of energy intake throughout the day is associated with the risk of obesity in adults. It has been studied that while energy intake in the morning was not associated with obesity, those who consumed ≥33% of daily energy intake in the evening were associated with a higher risk of overweight and obesity suggesting that eating more of the day's total energy intake at midday is associated with a lower risk of overweight and obesity [97]. Moreover, in other study, volunteers selected foods with higher caloric composition at dinner time than

breakfast time, suggesting a palatable preference that leads to an increase calorie intake [98]. Furthermore, eating patterns as frequency meals, breakfast skipping, or time-delayed pattern of eating relative to sleep, in this case patients with NES had an association with increased body mass index (BMI) [99,100]. In additional, experimental studies suggests that the timing of food intake is important in driving the obese phenotype. Otherwise, restricting feeding time, without calorie restriction for 8 h during the dark period in mice on a high-fat diet resulted in improvements in glucose and lipid homeostasis, alleviated inflammation, decreased body weight and attenuated the diurnal expression of clock genes *Per2, Bmal1, Rev-erb α* and *Cry1* in liver [101].

Genetic variance in clock genes may be important in meal timing as well. Timing of food intake was associated with genetic variance of the gene *Clock*. In an interventional loss weight study, volunteers were divided according to the timing of the main meal in early-eaters and late-eaters, those patients who ate at late hours lost less weight compared to early eaters despite consuming the same energy intake. The meal timing was associated to CLOCK rs4580704 SNP, having a higher frequency among late eaters [102].

Other clock genes polymorphisms including Per 2 have also been relate to obesity susceptibility through timing of food intake. Volunteers with this SNP used to skip breakfast and to display extreme snacking more frequently, being this associated with abdominal obesity [103].

Therefore, these findings suggest that the synchrony between metabolic and circadian processes plays an important role in energy balance and body weight control that will allow for clinical implications by developing strategies toward the composition and the timing of food for optimal body weight maintenance. Through nutrigenetics, behaviors may interact with our genes and may decrease the deleterious effect of one specific

risk variant. This is a novel and very promising area in obesity prevention and treatment.

1.2.2 Restricted feeding and food anticipatory activity (FAA).

Another dieting approach which is investigated is time restricted feeding; that is food provided *ad libitum* for about 3 to 5 h at the same time every day, usually at day time, without calorie restriction [104]. Restricted feeding in rodents originates an adjustment of locomotor activity to the diurnal feeding period within a few days called food anticipatory activity (FAA) that is an increase in locomotor activity 2-3 hours before presentation of distributed food in a circadian manner [104]. Thus, there is a hypothesis that rodents can learn and anticipate the time of regular feeding. Although the light–dark cycle is normal, mice show FAA during the daytime and reduce activity during the nighttime. According to recent studies, mutant mice lacking circadian clock function and SCN-ablated animals exhibit normal FAA, suggesting that FAA is independent of the SCN [105]. Thus FAA is driven by a food entrainable oscillator (FEO), which it is still controversial its location. It is thought to be in other regions in the brain, suggesting that circadian timing of food anticipatory activity involves an anatomically distributed population of FEOs, but their actual location is still unknown [106]. Evidence suggests that glucose or a palatable snack can induce the FEO and FAA. For example a palatable snack with different components as glucose and free fatty acids in the daytime could also elicit FAA and entrains daily oscillations of the clock gene *Per1* in other regions in the hypothalamus, which generate responses involved in homeostasis and reward, suggesting that the participation of *Per 1* in oscillatory processes is fundamental to food addictive behavior [107]. There is a hypothesize that central ghrelin signaling could be one of the multiple mechanisms to mediate this behavior [108].

1.2.3 Nutrient sensors and the circadian clock

Feeding behavior is a principal factor that plays a role in the organism nutritional status. It is demonstrated that different nutrient sensors are able to transmit information regarding the cellular nutrient status to the circadian clock. One essential is the AMP-activated protein kinase (AMPK) as a critical nutrient sensor, which is present in every tissue. Hypothalamic AMPK is important in regulating food intake in response to nutritional and endocrine food signals and is activated by fasting and low glucose levels [109]. On the contrary an abundance of nutrients, high levels of glucose reduces AMPK activity. In peripheral tissues activated AMPK switches on catabolic pathways, including fatty acid oxidation, and turn off anabolic pathways like lipogenesis or gluconeogenesis. Indicating an important role of AMPK in energy homeostasis [110]. AMPK display different actions on the circadian clock. Loss of AMPK signalling alters circadian rhythms in mouse hepatic clock genes. Likewise during fasting, AMPK phosphorylates a circadian repressor CRY and targets it for subsequent degradation and stability [111]. In addition, AMPK can phosphorylate casein kinase 1 ε (CK1 ε) which lead to phosphorylation of PERs [111]; thus preventing it from repressing CLOCK:BMAL1 target genes, such as *Rev-erbα*, *Per*, and *Cry*. A connection between AMPK and mTOR in the hepatic cell is of particular relevance, since this organ is uniquely designed to sense and respond to the availability of simple nutrients.

Another relevant metabolite is the cofactor nicotinamide adenine dinucleotide (NAD+). NAD is an essential coenzyme involved in many cellular redox reactions. NADs pathway involved a major enzymatic regulatory network in most cells [112]. NADs as indicators of energy metabolism can affect the activity of circadian clocks, nicotinamide phosphoribosyltransferase (NAMPT), a key NAD biosynthetic enzyme constitute a regulatory network. NAMPT expression levels increase under cellular stress and

nutrient restriction [113]. In addition, the SIRT1 a dependent NAD+ deacetylase, regulates transcriptions factors involved in pathways linked to energy metabolism, inflammation, diabetes, ageing and stress. SIRT1 is a member of the sirtuin family plays a role in all tissues and it is considered a sensor of metabolic changes that allow to cells surviving in situation of diminished nutrient availability [114], which is induced by acute nutrient deprivation or calorie restriction. Circadian oscillations in NAD+ levels drive SIRT1 rhythmic activity [115]. NAMPT and NAD+ directly regulate rhythmic expression of genes *in vivo* by binding to *CLOCK/BMALI1*. SIRT1, in turn, is recruited to the *Nampt* promoter along with CLOCK/BMAL1. The regulatory region of the Nampt gene contains two E-box promoter elements that bind to *CLOCK/BMAL1* and its expression is controlled by this circadian heterodimer [116,117]. Transcriptional loop of the circadian clock and the enzymatic feedback loop of the NAD+ pathway show this bidirectional relation. In fact, altered rhythmic levels of NAD+ disrupt behavioral activity and metabolism in mutant mice deficient in the NAD hydrolase, CD38 [118]. In addition SIRT1 is expressed in a circadian manner, is likely to have different functions in each tissue and is essential to circadian transcription in a gene specific manner of core clock genes, *Clock*, *Bmal1, Rory, Per2 and Cry1* [117]. In addition, several transcription factors controlled by SIRT1 are involved in cellular response to stress and nutrient metabolism such PGC1 alpha, the master regulator of mitochondrial biogenesis, which has also been shown to regulate the circadian rhythm *Bmal1* and *Rev-erbα*, through co-activation of the ROR family of orphan nuclear receptors [119]. Likewise there is a coupling between AMPK and SIRT1. For instance, AMPK enhances SIRT1 activity increasing NAD+ levels and they also regulate each other [120]. **(Figure 2)**. These data emphasize the importance of nutrient sensing signalling between the circadian clock and metabolism. A principal objective of future investigations is to outline how nutrient

signals interact to maintain alignment of metabolic rhythms in a tissue specific way and whole organism.

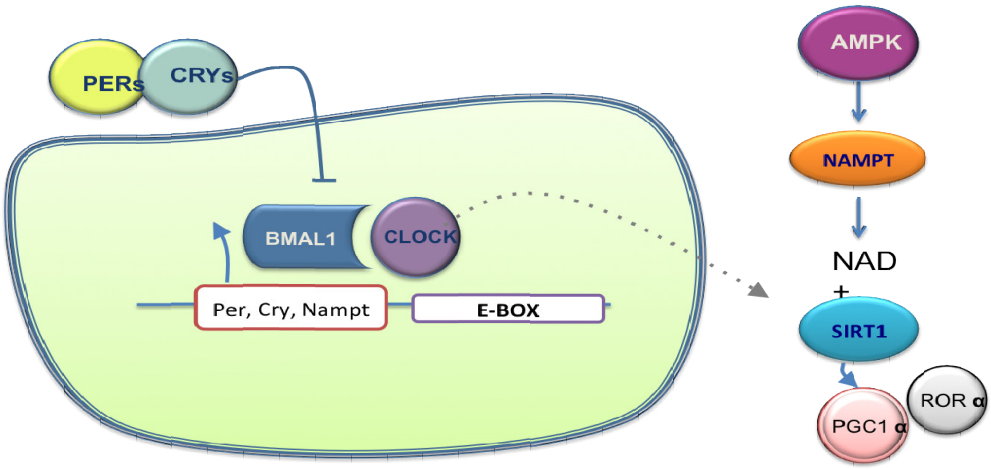


Figure 3. Integration of circadian clock and energy metabolism.
Nutrient sensors AMPK and SIRT1 are regulated in a circadian manner, AMPK activation leads to an increase in NAMPT and NAD+ levels, which, in turn activate SIRT1. This activation leads to PGC-1α deacetylation and activation which is required for RORα. These enzymes feedback to regulate circadian genes. Expression of Bmal1 is also regulated by PPARα, which is phosphorilated by AMPK. (Adapted from Froy *et al J Clin Sci 2011*).

1.2.4 Nutrient entrainment of the master clock

Although the light/dark cycle is clearly a major synchronizer of the core clock, other environmental factors can also influence the central clock. Diverse studies suggest that the phase of the central clock might be sensitive to nutrients and different feeding regimens. For example, food calorie restriction (CR), that is a reduction in the daily calorie intake without malnutrition has been shown to entrain the SCN clock [20, 121].

In contrast, meal time as modulated by temporal restricted feeding, has much less synchronizing influence on the SCN, except when food has either hypocaloric content or high palatable properties [122]. In combination a hypocaloric diet with timed restricted feeding lead to alterations in the diurnal expression patterns of *Per2* and *Cry1* in the SCN, as well as phase advances in locomotor activity and levels of melatonin [123].

In addition, glucose is capable to induce cellular circadian rhythms in SCN. For example parenteral nutrition of glucose during the inactive phase in rats shifted *Per2* expression levels in the SCN and in the liver in opposite directions [124]. Indicating that glucose could be a strong entraining signal for the SCN, but it also suggests that glucose is able to differently affect central and peripheral clocks.

The quality of nutrition can also change eating behavior and affect the central clock. High-fat diet feeding, particularly high intake of saturated fat modifies circadian synchronization to light in the SCN, increased locomotor activity and altered body temperature rhythms [125].

In addition, a high-fat diet lengthens the free-running period in male C57BL6/CJ mice under constant darkness (DD) conditions and increase food intake during the normal rest period under light-dark conditions [126]. Entrainment of SCN by a daily palatable diet has been observed in rats fed ad libitum. In constant darkness, animals received a palatable meal for 6 weeks; the diet entrained the circadian rhythm of locomotor activity. Neurons in the SCN respond to light during subjective night with an expression of the immediate early gene *c-fos* in the SCN the expression of *c-Fos* and *Per1* were in phase with the daily palatable meal [127]. The effects of fatty acids on the central clock could be through many feedbacks to the central clock, since the SCN receives information from the peripheral organs through different mechanisms, but the exact pathways are not known.

1.2.5 Nutrient entrainment of peripheral clocks.

Feeding is considered one of the most important external synchronizers or Zeitgebers for peripheral oscillators. Feeding and components of food, amount and changes in feeding time can reset peripheral clocks. In contrast to calorie restriction, which affects the clock in the SCN, time restricted feeding can entrain peripheral clocks. Changes in clock gene expression in responses to restricted feeding could vary depending of the gene studied. Diverse studies in mice have indicated for example that the liver clock is easily and strongly entrained by food [128] (**Figure 3)**. The study of these processes reveals different and wide physiological mechanisms that emphasize the relevance of food for rhythmic functions. For example, feeding time regulates the phase and levels of triglycerides in the liver of wild type and knockout mice of *Per 1* and *2*; under night time restricted feeding, and even in the absence of a functional *Per 1* and 2, feeding entrains the liver clock and maintain rhythmic oscillations [129].

The case of fat as a component has been studied through rodent models of diet induced obesity. Rodents consuming a high fat diet (HFD) *ad libitum* disrupts normal circadian oscillations of clock genes and clock-controlled metabolites and transcripts in the liver after only 3 days of HFD consumption and appeared to be reversible when mice is subjected to a normocaloric diet. The authors proposed that these HFD-mediated alterations in metabolism are linked to impaired CLOCK-BMAL1 chromatin recruitment. Demonstrating the acute metabolic consequences of HFD on the mouse liver [130]. Likewise HFD intake *ad libitum* induced a long, free-running period of locomotor activity rhythms and decreased the amplitude of clock or clock-controlled gene expression rhythms in the hepatic and adipose tissues [126].

An issue of importance is the amount of sugars consumed, similar to fat intake, glucose intake might be important for entraining the circadian clock. Glucose metabolism is highly circadian and depends on carbohydrate constitution [131]. High dietary fructose

intake is correlated with diabetes and cardiovascular disease in rodents and humans [132,133]. It has been investigated in mice fed a 8 weeks high fructose diet restricted only during the resting phase, animals gained more adipose tissue and body weight, showed increase insulin and leptin compared with night time-restricted access. Suggesting that the timing is critical in the response [134]. On the other hand the oral intake of sugars plus proteins can entrain clock genes in liver. Intraperitoneal injection of amino acids combined with glucose delayed the phase of clock genes *Per1* and *Per2* in liver [135]. Likewise, it was shown that carbohydrate intake alone consisting in fast absorption carbohydrates such sucrose and glucose, has only a minor phase resetting effect, while complex foods like high digestible carbohydrates have show much stronger effects. These results suggest that circadian clock is affected depending of the type of carbohydrate composition and indicating that a balanced proportion of macronutrients are required for proper entrainment of the circadian clock [136]. In addition to the proportions of dietary energy coming from the macronutrients influencing peripheral clocks; higher-protein, lower-carbohydrate diet phase advances of clock and increases the overall levels of *Bmal1* and *Cry*, PEPCK and glucose-6-phosphatase (*G6Pase*) in the liver and kidneys of high protein fed mice [137]. There are also several non-essential dietary compounds that have been shown to influence the circadian system. Alcohol seem to alter clock gene expression of *Bmal1, Clock, Cry1, Cry2, Per1*, and *Per2* in the liver of mice, and daily locomotor activity [138,139].

Another interesting adaptation that has been studied is the divided meals to rodents during the day to reflect a human diet. The regime consisted of six meals per day at a 4-h interval with a 20% food restriction of the amount of food consumed ad libitum per day, using an in vivo imaging system in PER2: Luciferase knock-in mice. The first meal following a long fasting period provided an important synchronizing signal in liver and kidney. Peripheral clock phases in vivo were unaffected by different feeding

frequencies as long as the feeding occurred at equal intervals, whereas phase changes were observed when meals were given at unequal intervals per day. Thus, peripheral tissues are entrained dependently of the quantity of food and the time between meals [140]. While alterations in the light and dark cycles disturbs the circadian system, another schedule protocol of 6 meals per day at equal intervals under short-day, medium-day and long-day photoperiods, in *in vivo* PER2:Luciferase mice, was found that this regime reverse alterations in the phases of peripheral clocks in liver and kidney when environmental changes in light occurred [141,142].

Clock genes in humans seem also to be responsive to macronutrient content. In a study designed to check the effects of a low carbohydrate/high fat diet compared with a low fat/high carbohydrate diet on humans, it was found that a change from a low carbohydrate/high fat diet to a high fat/low carbohydrate diet produced a phase delay in salivary cortisol levels and altered gene expression of PER1, PER2, PER3 in peripheral tissues, as measured by gene expression in circulating monocytes [143].

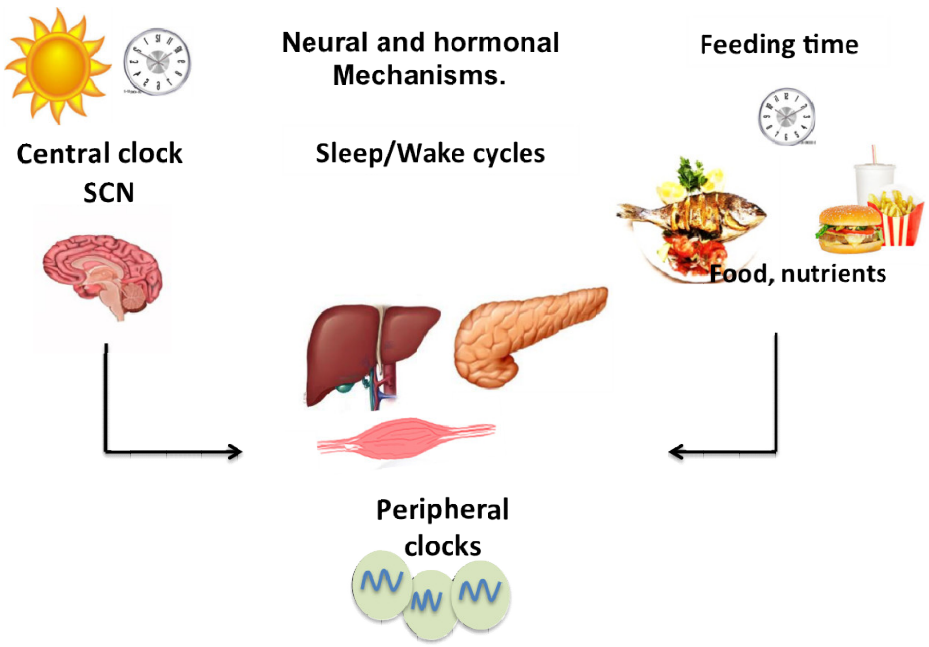


Figure 4. Central and peripheral clocks.
The master clock in the suprachiasmatic nucleus (SCN) of the hypothalamus drives sleep/wake cycles, locomotor activity, circadian feeding pattern and synchronizes many

peripheral clocks through multiple neuroendocrine mechanisms. Feeding time and nutrients affect either the SCN or peripheral clocks. (Adapted from We *et al J Nutr Sci Vitaminol*, 2015).

Modulating the external factors like time of food ingestion, quality of food, macronutrients distribution could lead to changes the entrainment of the endogenous clock with different dietary components [144]. More studies are drawing attention to which nutrients could significantly affect the circadian system. Nutritional ingredients with functional properties such as caffeine, resveratrol, vitamins, dietary proanthocyanidins, fish oil, have been reported to induce changes in the circadian clock [145–149]. So that functional food with beneficial effects could have an impact to the biological clock is promising for further research.

1.2.6 Taurine: An important aminoacid for metabolic health.

Taurine (2-amine ethanesulfonic acid), is the most abundant intracellular free amino and sulfonic acid in the body. The average amount in the body is approximately 560 mmol; around 70g in a 70 Kg- human adult, which is found in many tissues, including brain, retina, liver, pancreas, kidney, heart and adipose tissue [150]. Taurine is a conditionally essential amino sulphonic acid, not incorporated to protein synthesis, but it plays an important role in humans during development [151] due to the different physiological effects in the body [152]; different field of research has been focused in the study of taurine actions. *In vivo* studies have demonstrated that low levels of taurine are associated with various pathological alterations.

Today it is well known that taurine has many biological and physiological properties, such as anti-oxidation, anti-inflammation, osmoregulation, cell membrane stabilization, detoxification, modulation of cellular calcium levels and neuromodulation [153].

1.2.6.1 Taurine and nutrition

Taurine can be endogenously synthesized from methionine and cysteine, and is also provided by diet. The biosynthesis in vivo varies differently between species, having a high capacity in rodents and a moderate efficiency in humans. The key enzymes in the taurine biosynthetic pathway are cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD) mainly present in brain, liver, kidney and pancreas. CDO catalyzes the first step of the major pathway for cysteine catabolism and has a critical role in determining the flux of cysteine between cysteine catabolism and taurine synthesis [154]. For example, *Cdo* knockout mice has a blockage in cysteine metabolism, which decreases taurine biosynthesis, also these mutants display altered energy balance, increased hepatic levels of stearoyl-CoA desaturase 1 (*Scd1*) and excessive production of hydrogen sulphide (H_2S) that impairs mitochondrial-oxidation of fatty acids [155]. Taurine is biosynthesized in different tissues, being the liver the main organ capable of synthesizing taurine. Other tissues as white adipose tissue, pancreas, kidney, retina have lower biosynthetic capacity [156]. Many protective functions of taurine on the liver damage have been reported. Taurine has been found to have preventive effects on the development of hepatic steatosis induced by a high-fat diet in rodents [157]. Accumulation of taurine is mostly through dietary sources and via an active transport system. After absorption in the intestine, mediated active transport in the brush border membrane moves taurine to enterocytes, which deliver it to the portal vein. The contribution of taurine transport depends upon the expression level and regulation of a taurine transporter (TAUT), which in turn responds to the concentration of taurine in cells [154,158]. Recently, it has been studied taurine deficient KO mice whose mutation include the taurine transporter (*TauT*), this mutant has wide alterations due to a taurine depletion and a decreased taurine uptake; for example alteration in KO *TauT* mice shows a phenotype with decreased body weight, cardiac dysfunction and skeletal

muscle atrophy, implying that taurine deficiency in some way affects different processes, also these mutants presented deleterial effects on brain, development of hepatic damage like hepatitis and liver fibrosis, reduced exercise capacity and retinal degeneration at an early age [159]. In this manner defective taurine transport alters taurine homeostasis and leads to pathophysiological conditions.

1.2.6.3 Taurine intake and food sources

Daily quantity of taurine intake range between 200-400 mg/d and an increased dietary intake of taurine has been associated with multiple beneficial health outcomes [160,161]. Estimates of dietary intake of taurine vary differently. Taurine values from the same food sources are fairly consistent across different studies. Despite of all the studies realized there are no existing recommended dietary allowances (RDA). Table 1 shows the food sources where taurine is found, specially is rich in many kinds of seafood. Taurine has not been detected in plants and plant products.

Table 1. Taurine average content in food. [162]

Taurine content in food	mg/100g
Cod	31
Octopus	388
Pork meat	61
Salmon	94
Sardine	122
Squid	356
Turkey	39.8
White fish	113.9

1.2.6.2 Taurine, aminoacids and circadian rhythms

The role of amino acids on circadian rhythms has not been well investigated. A combined injection of glucose with all essential amino acids induced rapid changes in clock gene expression of *Per1* and *Per2* in mouse liver [135]. Circadian levels of tryptophan and alanine were altered in mice with altered NAD^{+}. Suggesting that these amino acids constitute a nutritional signal for the liver [163].

It was shown that taurine is present in high levels and exhibits circadian rhythms in the pineal gland of rats [164]. Likewise it has been found that taurine levels are altered during sleep deprivation period in rat and humans [165,166], but the ethiology and implicated mechanisms are unknown.

1.2.6.3 Taurine and metabolic diseases

Epidemiological data and animal studies suggest that taurine consumption has beneficial effects in diseases like obesity and diabetes. First plasma taurine concentrations are found to be low in patients with diabetes and obesity. The exact mechanism it is not known, and could be due to different factors. One possibility is suggested by a decreased net intestinal absorption and the renal excretion rates of taurine, which are high due to alterations in the taurine transporter during diabetes and decreased levels of the rate-limiting enzyme of taurine biosynthesis CDO in adipose tissue during obesity [167], also acute hyperglycaemia, reduces the expression of Taut mRNA and protein in different cellular models [168]. Hyperglycemia has been considered as the etiological source of diabetes complications. In animal models of type 2 diabetes and obesity taurine improves insulin resistance and decreases high glucose concentrations protecting beta cells and promoting insulin secretion [169], also suppressive effect of taurine against oxidative stress is associated with various pathways in diabetic condition such as the modulation of mitochondrial calcium and

reducing glycation and end products (AGEs) [170], against high glucose exposure. These findings indicate that the hypoglycemic properties of taurine are mediated by different mechanisms in whole organism; modulation of insulin sensitivity and insulin secretion for the control of glucose homeostasis, and via antioxidant an anti-inflammatory mechanisms alleviating diabetic complications. It has been proposed that taurine acts in peripheral tissues and CNS in obesity-linked complications [171]. Although the mechanisms by which taurine ameliorates obesity are at present unclear, it acts through multiple mechanisms regulating energy expenditure, affecting directly lipid metabolism; in adipose tissues, inhibition of oxidative stress and alleviating the adipose tissue inflammation in diet-induced obesity [172]. Despite the large evidence in experimental studies, clinical trials still are insufficient to translate this efficiency and protection by taurine in diabetes and obesity.

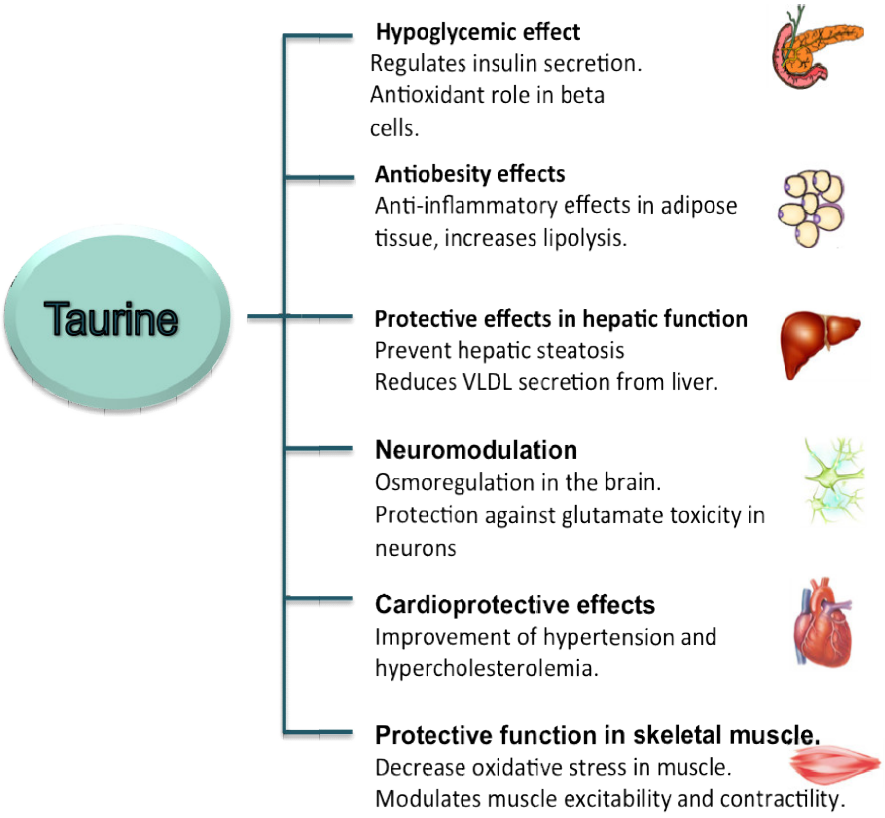


Figure 5. Whole organism effects of taurine.
(Adapted from De Luca *et al* *J Transl Med* 2015).

1.3.1 Circadian disruption and metabolic diseases

The obesity epidemic has evolved into one of the biggest global health threats of the 21st century, affecting approximately 400 million people worldwide and with its increasing prevalence; obesity is a significant global health problem. It is a well-recognized risk factor for metabolic and cardiovascular disease and arises from an imbalance between energy intake and expenditure on a background of complex genetic susceptibility [173]. The clear causes of obesity include genetic factors, excessive food intake and inadequate physical activity. However, other lifestyle related factors that have been implicated in obesity such as sleep duration, eating habits, and shift work have not always received enough attention until few years ago [174]. Research towards understanding how obesity predisposes to chronic metabolic diseases like metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular diseases. T2DM is considered a multifactorial disease in response to genetic factors, varying degrees of overnutrition, inactivity, consequential overweight or obesity and insulin resistance. Obesity has been found to contribute to approximately 55% of cases of T2DM [175]. According to the International Diabetes Federation, 382 million people (8.3%) worldwide currently have diabetes; the number of people with T2DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. This number is expected to increase to 592 million, implying that there will be around a 50% increase in diabetes by 2035 [176].These factors include pancreatic beta-cell dysfunction, abnormal adipogenesis and absence of adequate insulin responsiveness, genetic susceptibility, excessive food consumption and/or high calorie food intake. Both beta-cell failure and insulin resistance contribute to pathogenesis of diabetes; this leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. The involvement of impaired alpha-cell function has recently

been recognized in the pathophysiology of T2DM these dysfunctions precede the development of overt hyperglycemia [177].

Various types of circadian disorders have been correlated with obesity and the onset of T2DM. As mentioned before, recent studies have demonstrated that diet induced obesity like HFD disrupt circadian rhythms. HFD in mice alter levels of leptin and glucose during both the light and dark periods. The changes in behavioral rhythmicity correlates with disrupted clock gene expression within hypothalamus, liver, and adipose tissue, as well as with altered rhythms of hormones that contribute to these chronic degenerative diseases [126,178]. Obese diabetic mice showed altered pattern of *Rev-erb α, Dbp* and *Per2* genes in visceral fat that was associated with local inflammation and impairment of AMPK, PPARγ and PEPCK [179].

Maternal obesity also influences in the risk of obesity in the offspring. More specifically, recent studies suggest that circadian rhythms are affected since the gestational exposure to dietary manipulations; in this case, pregnant rats subjected to an enteral diet to induce obesity from preconception to birth, the offspring of obese rat gained more body weight and fat mass when fed a high fat diet, resulting in the impairment of clock genes *Clock, Rev-erbalpha, Bmal1* and metabolic genes *Pparα* and *Sirt1* in the liver prior to obesity development [180]. Although studies in mice have demonstrated that diet-induced obesity and type 2 diabetes caused by chronic high-fat diet consumption eating behavior, and daily pattern of locomotor activity are partially or completely reversed by feeding mice low-fat diet even in humans [181].

1.3.2 Circadian Disruption and Environmental factors

The growing evidence about the circadian regulation of tissue metabolism supports the idea that coordination between the internal clock and environmental cycles is important

for body homeostasis. Normal fluctuations in body weight associated with seasonal changes in day length have been observed in different mammals. In rodents decreasing the length of the light phase results in significant weight gain [182]. With actual life and modern technology, night shift and rotating shift work have become more frequently common; such changes alter activities but also physiological and molecular rhythms in the body. Crucial demonstration that these alterations contribute to metabolic modifications has been observed in night shift and rotating shift workers experiments. Shift work is an example of circadian disruption, by altering the timing of light exposure, meals, activity, and sleep. One of the primary effects on circadian desynchrony from shift work is a reduction in total sleep time. In this point association between cardiovascular disease, increase body weight, and altered cholesterol and triglycerides levels have been studied [183]. Epidemiologic studies indicate that shift work is associated with an impaired metabolism and increased risk of obesity and diabetes. The restriction of sleep in normal patients contributes to the development of prediabetic metabolic state and insulin responses to hyperglycemia characteristic to insulin resistance. It has been demonstrated that chronic sleep restriction impairs glucose regulation by decreasing insulin secretion and increasing plasma glucose concentrations after a meal, with a reduction in the resting metabolic rate [184,185]. In addition, with a short-term circadian misalignment in healthy volunteers, their rhythm was shifted gradually by 4 h a day, through implementation of a 28-h instead of a 24-h day. Subjects also exhibited alterations in metabolism, including increased glucose and insulin levels, decreased leptin levels and elevated arterial pressure [186, 187].

In an interesting study, sleep was restricted by delaying bedtime and advancing wake time each by 2 h within 5 days of insufficient sleep, as result, the volunteers increased total daily energy expenditure, energy intake, especially during the night leading to weight gain [188]. Since feeding time is considered one of the most important external

synchronizer, unusual feeding times are likely to be one of the risks factors to health in modern people. These results suggest that increased food intake during sleep loss appears to be a physiological adaptation to confer the body the required energy to keep prolonged periods of sleeplessness. Previous studies have reported that obese patients were sleepier during the day and more likely to present disturbed sleep at night compared with normal weight patients [189]. Although the mechanisms of how sleep alteration and diabetes risk are not fully understood. Different pathways have been postulated implying the impact on glucose tolerance. The idea that circadian misalignment would increase the risk of diabetes and metabolic dysfunction adapts well with clinical data and a large body of experimental evidence. Most likely, alterations to the sleep–wake cycle influence the central clock, which could alter peripheral clocks. Altering feeding patterns could result in desynchronization between the central and peripheral clocks, which will eventually alter body metabolism. Thereby the relationship between circadian disruption and metabolic pathologies seems to be in different directions in humans, suggesting that circadian disruption may lead to a vicious cycle that contribute to increase and continuing progress of metabolic disease.

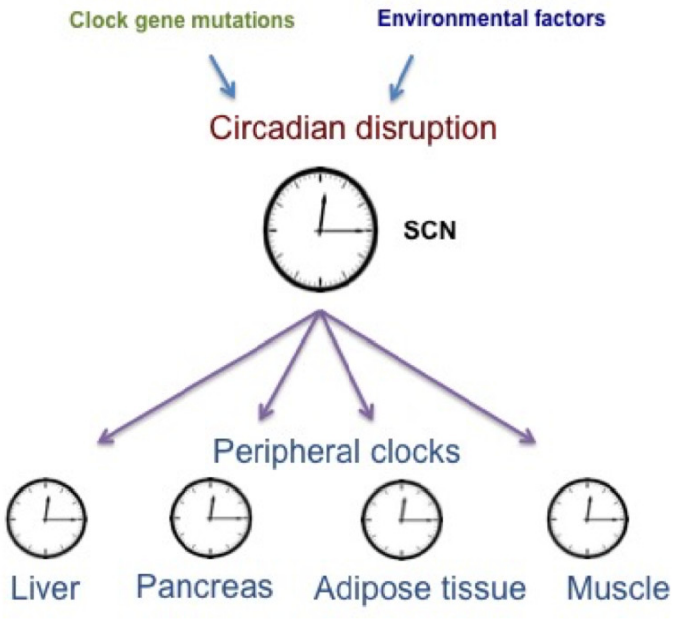


Figure 6. Effect of the SCN on peripheral clocks
The suprachiasmatic nucleus (SCN) resets signals in clock genes in peripheral tissues, such as liver, pancreas, adipose tissue and muscle. Disruption of circadian clock by genetic and environmental factors is associated with different diseases. (Adapted from Preuβner *et al* Eur J Physiol 2016).

1.3.3 Circadian Disruption and Genetic factors

Circadian disruption can be produced by alteration of the core machinery of the circadian clock. Broad evidence in the study of genetic mutations of clock genes couples the connection to metabolism. Mutations in the core clock genes are known to produce different alterations in metabolic pathways.

A deficiency in a particular clock protein results in two different conditions; disruption of circadian rhythms and development of a primary disease.

Mice mutants of *Clock* are obese resulting in metabolic syndrome, these animals present hyperlipidemia, hyperglycemia and hyperleptinemia [190]. Mutations in the heterodimer CLOCK /BMAL1 in the pancreas develops diabetes mellitus due to beta cell failure and loss of glucose stimulated insulin secretion [191]. In the same way recently it was shown that Clock/Bmal1 regulates insulin sensitivity in muscle via SIRT1 [192]. The nuclear receptors REVERBs are known to be crucial regulators in metabolism. Specially REV-ERBα is highly expressed in most organs, and has different roles in each one; besides its key role in the regulation of the transcriptional feedback loop, the wide study of its functions, involves functions from cellular differentiation and metabolic regulation. *Rev-erbα* mutants reduced physical activity associated with hyperglycemia and present higher triglycerides levels [193]. Also it was shown that Rev-erb agonists reduced fat mass and improved dyslipidemia and hyperglycemia [194]. Another relevant regulators are the family of *Cry* genes. *Cry 1/2* $^{-/-}$ mutant mice rapidly gain weight, and presents hyperinsulinemia, higher vulnerability of lipid storage and adipocyte

hypertrophy when challenged a high fat diet [195]. In addition deletion of *Cry1/2* is sufficient to increase the stress levels of cells leading to constant expression of inflammatory cytokines and causing a low-grade chronic inflammatory [196]. It has been shown that both *Per1 / Per2* $^{-/-}$ mice show weight gain relative to wild-type controls under a normal light/dark cycle [56]. *Per1/2* disrupted macrophages similarly exacerbate inflammatory responses and decrease insulin sensitivity [197]. In addition m*Per2*-deficient mice elevated plasma insulin levels, become obese, showing an increasing food intake during day and night when mice eat a high fat diet [198, 199]. Thus different studies based on systemic genetic deletion of clock genes have demonstrated the involvement in the regulation of metabolic homeostasis and targeting the circadian clock would seem an important and relevant therapeutic aim.

In humans, polymorphisms (SNPs) in some clock genes have been correlated with metabolic risks. Polymorphism of the Clock gene rs1801260 has been reported to have a role in the development of diabetes in humans, which is associated with a low or high prevalence of metabolic syndrome depending their haplotype. Circadian clock variants have also been found to correlate with body mass index (BMI), weight loss, sleep duration and total plasma cholesterol in obese humans [200]. Different polymorphisms haven been discovered related with CLOCK and metabolic syndrome as well. In the SNP rs4580704 minor allele carriers had a lower risk of hypertension and higher insulin sensitivity [201]. In relation to dietary responses the gene PER2 is associated with weight loss. Two different SNPs, which are associated with abdominal obesity and linked to eating behavior phenotypes. Moreover carriers of the minor allele in these SNPs presented increasing overeating by higher snacking of carbohydrates. They also had a bigger probability of being obese and of dropping out a weight loss treatment [202]. Suggesting an important function of PER2 in feeding behavior and success of

behavioral interventions aimed to lose weight could be predicted in part by genetic variance of clock genes.

On the other hand, REV-ERBα gene polymorphisms seem to modulate adiposity. The rs939347 SNP could modulate body fat mass that has a higher frequency in men, being associated with obesity in spanish obese male population [203]. In addition rs2071427 REV-ERBα SNP has been found to be related to obesity in young people [204]. However few studies assessed the impact of genetic polymorphisms in REV-ERB α. Although there is no complete mechanistic explanation yet for these observations, they clearly point to adverse effects of an alteration between circadian rhythmicity with different features of obesity as well as diabetes.

Table 2. **Metabolic alterations in clock gene mutations**. (Adapted from Eckel-Mahan *et al Physiol Rev* 2012

Clock gene mutations and metabolic phenotypes	
Clock Gene	**Metabolic phenotype in mice**
Clock -/-	Obesity, altered renal function, metabolic syndrome.
Clock Δ19	Impaired glucose sensitivity, obesity, reduced islet size and proliferation.
Bmal1 -/-	Reduced activity, accelerated aging. Fasting hypoglycemia (liver KO). Diabetes (pancreas KO).
Rev-erb α -/-	Increased adiposity, altered hepatic TGA levels, high plasma LDL. Increased plasma glucose and TGA.
Per1 -/-	Arrhytmic locomotor activity, increased urinary sodium excretion.
Per1^{S714G}	Increased food intake, rapidly develop obesity on a high- fat diet.
Per2 -/-	Altered lipid metabolism, hyperinsulinemia.
Per1/Per2 -/-	Decreased activity, increased body weight, altered TGA levels.
Cry1/Cry2-/-	Hyperglycemia, glucose intolerance, high levels of corticosterone, altered light response.
Rorα -/-	Altered plasma TGA and HDL.

1.4. Role of circadian rhythms in glucose homeostasis.

The circadian system has been shown to regulate glucose metabolism; time of day dependent oscillations in glucose metabolism are seen in healthy humans. In addition to feeding/fasting cycles, metabolically relevant circadian fluctuations involve glucose and insulin. Oral glucose tolerance is impaired in the evening compared to the morning; an effect believed to be due to a combination of both decreased insulin secretion and altered insulin sensitivity in the evening. The 'dawn phenomenon' refers to an elevation in blood glucose levels prior to the onset of the active period that has been well-documented having a peak in the morning over the night time hours an event known as dawn phenomenon [205].

Likewise oscillations in blood glucose levels continues in rats with lesioned SCN, independently of the feeding activity in rat, this is regulated by a through direct mechanism of the SCN that control glucose concentrations; at the same time at the end of the light, there is a rise in basal glucose concentrations, the SCN stimulates endogenous glucose production, thereby increasing glucose concentrations and compensating for the high glucose uptake, therefore prepares and adapts the organism for the activity period by increasing plasma glucose concentrations and by making the tissue more tolerant to glucose [15,206].

Both insulin dependent and insulin independent glucose disposal shows daily variations in humans and rodents. CLOCK mediated regulation *in vitro* studies of insulin secretion from isolated perfused rat pancreatic islets shows an endogenous circadian oscillator is located within the pancreas [207]. Also studies in humans confirmed the circadian rhythms in insulin secretory response with different levels of glycemia, which becomes rising during the day and falling during the night [208]. While this normal circadian insulin secretion is altered in first-degree relatives of type 2 diabetic patients

[209]. Suggesting a probability that may serve as a biomarker of beta cell dysfunction in type 2 diabetes. Thereby since at the central level there is a direct influence on glucose metabolism through the autonomic nervous system and hormonal outputs, also peripheral clocks influence glucose metabolism, it is not clear yet how are these interactions at different levels of circadian regulation with each other to accomplish optimal glucose regulation.

1.4.1 The pancreatic clock

Glucose homeostasis is approached through the regulated coordination of endogenous and exogenous glucose and it's sustaining and utilization is necessary for daily functioning. Pancreatic islets, which are small, island-like structures within the exocrine pancreatic tissue and consist of different endocrine cell types that are clustered together. These cells include beta cells, alpha cells, delta cells and PP cells (pancreatic-polypeptide) and ghrelin producing cells to maintain the homeostasis playing a pivotal role in the systemic regulation of metabolism [210]. Insulin and glucagon lowers and increases plasma glucose levels respectively and are secreted reciprocally in response to variations in plasma glucose levels [211]. Insulin predominates in the fed state, promoting glucose uptake by its target organs whereas glucagon mobilizes hepatic glucose in fasting to ensure the sustaining levels of glycemia [212]. Under physiological conditions the beta cells respond to a meal with the immediate secretion of insulin. Insulin reciprocally regulates alpha-cell glucagon secretion [213]. As insulin gets into circulation decreases hepatic fuel production by counteracting glucagon action and also enhancing glucose uptake by skeletal muscle and adipose tissue [214].

1.4.2 Insulin secretion

Endocrine cells secrete their respective hormones in response to external signals, such as nutrient intake or stress, via humoral, neural or hormonal signaling pathways. The underlying molecular process that translates the stimulus into the actual hormone release is called stimulus-secretion coupling. In the beta-cells, the principal stimuli for insulin release are increased blood glucose levels following a meal. The circulating blood glucose is taken up by the facilitative glucose transporter GLUT2, which is located on the surface of the beta-cells [215]. Once inside the cell then is phosphorylated by glucokinase and glucose undergoes glycolysis and metabolized, the ATP produced triggers a cascade of signals needed for glucose stimulated insulin secretion (GSIS) [216]. This increased ratio of ATP/ADP leads to close the K_{ATP} + channels that blocks potassium exit from the cell and depolarizes the cell membrane. L-type voltage-dependent calcium channels are opened, and the rise of intracellular calcium concentrations triggers the release of insulin from granules containing a pool of insulin vesicles [217]. Hyperglycemic clamps and experiments in isolated pancreatic islets have demonstrated that glucose induces insulin secretion in a biphasic pattern, with the first phase peaking around 5 minutes after the glucose stimulus with the majority of insulin being released during this first phase [218]. Glucose-stimulated insulin secretion is a complex mechanism modulated also by several factors, such as amino acids, free fatty acids, incretins, hormones and neural inputs

1.4.3 Glucagon secretion

The production and secretion of glucagon are crucial mechanisms by which the organism prevents hypoglycemia. It is postulated that at decreased blood glucose levels, the regulation of glucagon by glucose is mediated too by the ATP/ADP ratio, this

proceed to closure of K_{ATP} channels and membrane depolarization through a combination of voltage-dependent $Ca^{2+,}$ Na^{2+} channels. The release of glucagon is triggered by Ca^{2+} entry through these voltage-dependent Ca^{2+} channels [219]. There are other mechanisms explaining how glucagon secretion is regulated. It has been proposed that glucose by sequestering Ca^{2+} into the endoplasmic reticulum could close the store-operated channels inhibiting glucagon secretion. The mechanism for the modulation of intracellular Ca^{2+} is still controversial and has been ascribed to a direct glucose effect, or to a paracrine effect related with beta cells. Potential paracrine regulators include GABA, Zn2+, somatostatin and insulin modulates glucagon secretion or inhibition [220].

This argues that the alpha-cells possess sort of intrinsic glucose-sensing which operates independently of paracrine signaling since glucagon is modulated in the range of glucose concentrations below 4-5 mM, however the mechanisms involved not completely clear.

At increased blood glucose levels glucagon secretion is suppressed and reduces its stimulatory effects on hepatic glucose production. High glucose inhibits glucagon secretion by slight sustained depolarization after closing K_{ATP} channels. The depolarization preferentially inactivates the Na^{+} channels thereby preventing Ca^{2+} influx through the P/Q channels and glucagon release.

In a diabetic state, the pathogenesis of T2DM is classically focused on insulin resistance and beta-cell dysfunction, an inadequate increased alpha-cell function and consequent hyperglucagonemia has been established as a contributor to hyperglycemia in diabetic patients, by stimulating hepatic glucose production [221]. Glucagon release in diabetes shows different abnormalities a hypersecretion during hyperglycaemia and a failure in the response to hypoglycemia. Thus a dysfunction

where total relative lack of insulin or insulin insufficiency is present in parallel with abnormal increase in glucagon secretion is a hallmark of T2DM.

1.4.4 Clock genes and pancreatic islets function

As mentioned before, this principal level of control on glycaemia by the islets of Langerhans depends mostly on the coordinated secretion of glucagon and insulin by the alpha and beta- cells. The endocrine pancreas contains its own circadian clock that oscillates over the course of the 24 h day and affects tissue-specific metabolic processes. Islet expression of *Clock, Bmal1, Per1, Per2* and *Rev-erbα* shows daily oscillations in mice and rats and humans [222,223]. The most convincing evidence that clock function within the endocrine pancreas impacts glucose homeostasis has emerged from studies in mice with tissue specific ablation of *Clock* and *Bmal1*. *Clock* mutants showed decreased expression levels of genes downstream of CLOCK: BMAL1 that comprises the core circadian loop, providing evidence for a self-sustained clock in endocrine pancreas [191]. The defects in clock mutant islets appear to result in impaired insulin secretion in response to glucose and other secretagogues and decreased islet proliferation. *Clock* mutant mice show impaired glucose tolerance with reduced stimulated insulin secretion and higher levels of glucose through the entire light/dark cycle. To determine in vivo relevance of the core clock machinery in pancreatic beta-cells, the authors generated pancreas-specific *Bmal1* KO mice. Despite normal locomotor activity rhythms, these animals displayed more pronounced hyperglycemia than systemic *Clock* mutant or global *Bmal1* KO mice; the precise molecular details remain to be studied. Providing evidence for a molecular regulator of pancreatic glucose-sensing and/or insulin secretion mediated by CLOCK and BMAL1 in pancreatic beta- cells [224,225].

BMAL1 and the circadian clock have been postulated to regulate oxidative stress in other peripheral tissues. Another interesting finding is that deletion of BMAL1 function in beta-cells leads to a ROS accumulation in beta-cells due to an impaired scavenging in mitochondria, which may contribute to the impairment of GSIS also *Bmal1* directly regulates the expression of the key antioxidant response regulator *Nrf2*. Thus this evidence suggest that circadian clock alleviate oxidative stress in beta cell dysfunction [226]. Another relevant regulator of insulin secretion is the nuclear receptor Rev-erbα, which is implicated in control of insulin secretion. Decreased levels of *Rev-erbα* lead to a reduction in beta- cell proliferation in primary beta-cells and beta-cell lines. It was also found that *Rev-erb α* regulates lipogenic genes in mouse islets. During adaptations in obesity authors found that leptin coordinate levels of *Rev-erbα* in beta-cells [227]. It seems the greater influence of disturbed clock mechanisms in the beta cells appears to be an impaired insulin release.

The clock gene *Per2* has been shown to regulate insulin secretion. In *Per2* knockouts animals plasma insulin levels were elevated which was associated with enhanced glucose-stimulated insulin secretion and with increased GLUT2 expression in pancreatic islets of *Per2* KO mice [198], but the precise role in beta cell is not known.

In addition, changes in the light–dark cycle in vivo entrain the phase of islet clock and while long term exposure to light disrupts islet circadian clock function by impairment of clock genes oscillations. [228].

Additionally to insulin, glucagon also plays a crucial role in regulating blood glucose homeostasis. Its secretion from the pancreatic αlpha cells has been shown to display diurnal patterns of secretion. It has been reported that feeding and the biological clock control 24-h plasma glucagon concentrations [229]. Melatonin receptors are expressed in alpha cells, and it has been studied that melatonin influence both glucagon expression and secretion in alpha cells in vitro and in vivo and affects the actions of glucagon on

hepatocytes [230]. In this manner circadian clocks contribute to an extended role in relation to the endocrine pancreas homeostasis, through regulation of endocrine factors like insulin and glucagon although limited information exists about possible role of clock genes on alpha cells. In humans studies of polymorphisms in the CLOCK gene showed that the molecular clock could perform a role in the vulnerability for type 2 diabetes, obesity and metabolic syndrome [231,232], whereas genetic variations in PER2 gene have been linked [198]. This evidence show that clock genes directly and indirectly affects metabolic homeostasis in humans.

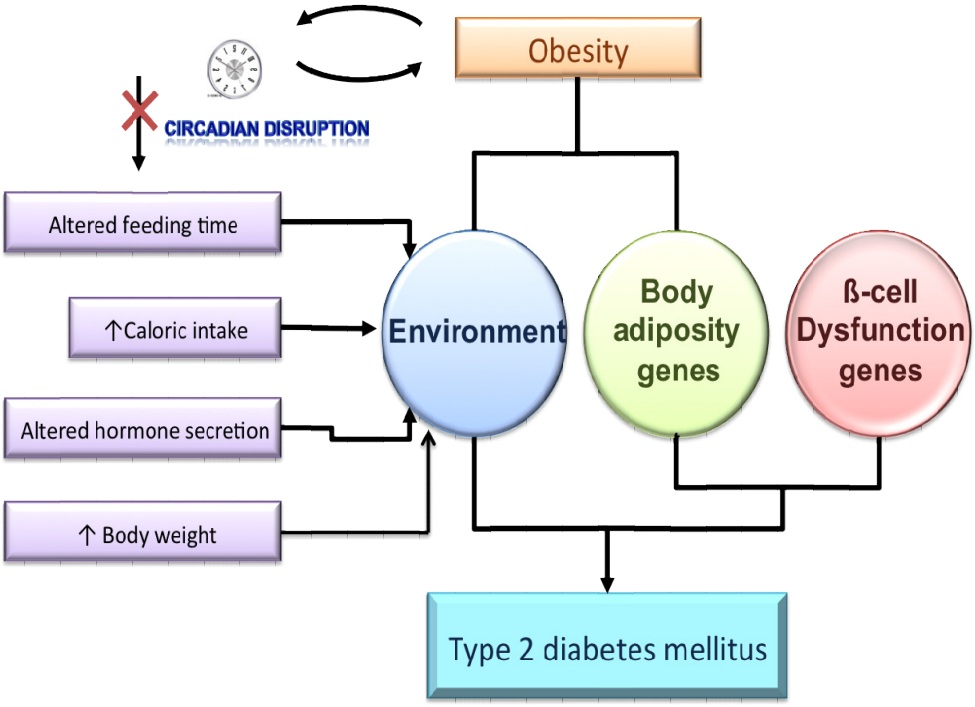


Figure 7. Circadian clock disruption
Effects of alter circadian clock functions in peripheral clocks in adipose tissue and pancreas creating an altered reciprocal cycle that disrupt behavior and metabolic activities leading to obesity and diabetes. (Adapted from Kahn *et al The Lancet* 2014).

2. HYPOTHESIS AND OBJECTIVES

2.1 Rationale and hypothesis.

Circadian rhythms are important for maintenance of systemic metabolic homeostasis, clock dysfunction by different factors like excessive caloric intake, altered feeding time and sleep disturbances correlate with higher risk of developing obesity and diabetes, likewise these diseases themselves promotes further circadian alteration. It is crucial to find approaches to maintain normal circadian physiology and as a strategy to treat obesity and diabetes. Recently research focused on diverse nutrients has demonstrated that are important factors to entrain the circadian clock with plausible efficiency in ameliorating circadian rhythms. In this point, the amino sulphonic acid taurine is considered to have a potent impact in different health benefits and protection in metabolic diseases.

Thus, we hypothesized that taurine by modulating circadian rhythms could prevent or ameliorate metabolic abnormalities caused by HFD feeding.

2.3 General and specific aims

General aim. **To find novel nutritional interventions to improve disturbances on circadian rhythms in an animal model of diet induced obesity.**

In order to assess our main hypothesis, we established the following specific aims:

Specific aims

A. To investigate the effects of taurine in glucose homeostasis in short term and long-term treatment in mice fed with high fat diet.

B. To determine if taurine treatment can ameliorate the circadian rhythms of metabolic parameters and daily hormone secretion in mice fed with high fat diet.

C. To check whether taurine treatment could modulate the 24 h pattern of clock gene expression in peripheral tissues.

3. MATERIALS AND METHODS

3.1 In vivo characterization

3.1.1 Animal model and experimental groups.

To assess the role of taurine on circadian rhythms, we utilized the following design: We used 8-9 weeks old male mice C57BL/6, at the beginning of the experiment. Animals were allowed to adapt to their environment for 1 week. Mice were fed ad libitum with chow diet or high fat diet (45% fat Research diets Inc. D12451). All protocols for animal use and euthanasia were reviewed and approved by the Animal Research Committee of the University of Barcelona and principles of laboratory animal care were followed, according to European and local government guidelines being approved this study (ID 5434).

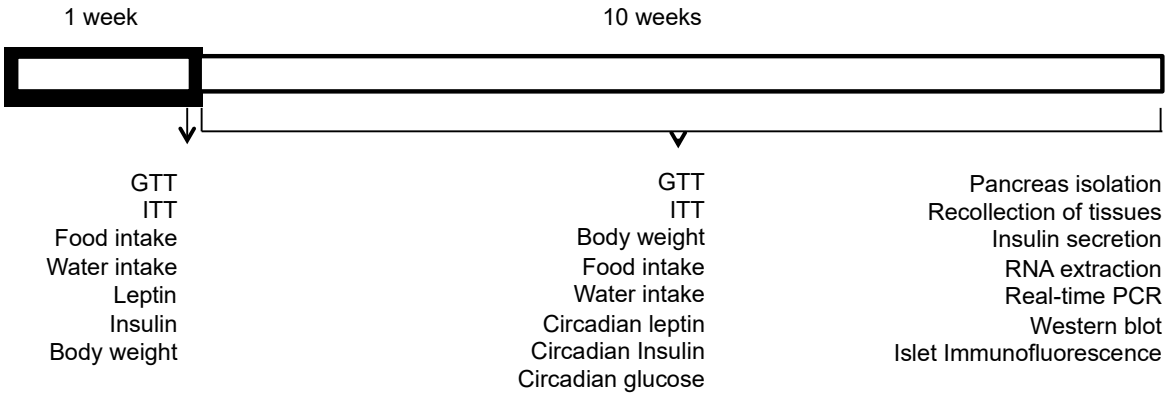
The experimental groups were divided in 4 groups: Controls fed with chow diet (C), controls fed with chow diet and 2% taurine (Sigma-Aldrich, St. Louis, MO) in drinking water (C+T), mice fed with high fat diet (HFD) and mice fed with HFD and 2% taurine in water (HFD+T). We used this dosage of taurine according to previous studies. Various reports have described the experimental use of taurine supplemented in drinking water in mice over the concentration range of 0.05%~5% [233–235].

Table 3. Nutritional composition of experimental diets

	Chow diet		HFD	
	g %	Kcal %	g%	Kcal %
Protein	14.3	20	24	20
Carbohydrate	48	67	41	35
Fat	4	13	24	45
Total		100		100

3.1.2 Study design

We performed first a preliminary study for 1 week of HFD and taurine treatment, to assess mice phenotype for a short-term intervention. Following this we assessed the effect of HFD and taurine in a long-term study of 10 weeks.



3.1.3 Preliminary Study 1 week

Mice were allowed free access to food and water, maintained a 12h light–dark cycle at 24°C and constant humidity in soundproof cages. Body weight, water and food intake were measured at the beginning and at the end of each week of intervention. Food intake was monitored by measuring the amount of food consumed from 8:00 to 20:00 (light cycle) and from 20:00 to 8:00 (dark cycle). Water intake was monitored by measuring the volume of water consumed at the beginning and the end of the treatment. IpGTT, ITT and insulinemia were measured. Blood samples were collected to measure plasma insulin and leptin during fed state. Insulin and leptin were quantified by ELISA (Mercodia Insulin, Uppsala Sweden and Crystal Chem, Harris County, TX, USA), respectively.

For this preliminary study we had measured only at one point of the day at 12:00. By the end of this week, mice were euthanized and visceral adipose tissue was weighed.

3.1.3.1 Metabolic tests

Glucose tolerance test

For an intraperitoneal glucose tolerance test (IPGTT), a solution of glucose (2g/kg body weight) was injected intraperitoneally in 6h fasted mice. Blood samples were collected from the tail vein before the injection at time 0, 15, 30, 60, 90 and 120 minutes with a microvette (Sarstedt). Glycemia was immediately measured using a glucometer with tests strips (Accu-Check; Roche Diagnostics, Madrid, Spain). Following immediate centrifugation at 4°C, plasma was separated by centrifugation and stored at 20°C for further measurements of insulin.

Insulin tolerance test.

For insulin tolerance test, 4h-fasted mice were injected intraperitoneally with 0.75 IU insulin/kg body weight (Actrapid, Novonordisk). The blood glucose concentrations were monitored prior to (0 min) and at 15, 30, 60, 90 and 120 min after insulin administration. Blood samples were collected before insulin injection (time 0) and at 15, 30, 60 and 90 min after insulin administration.

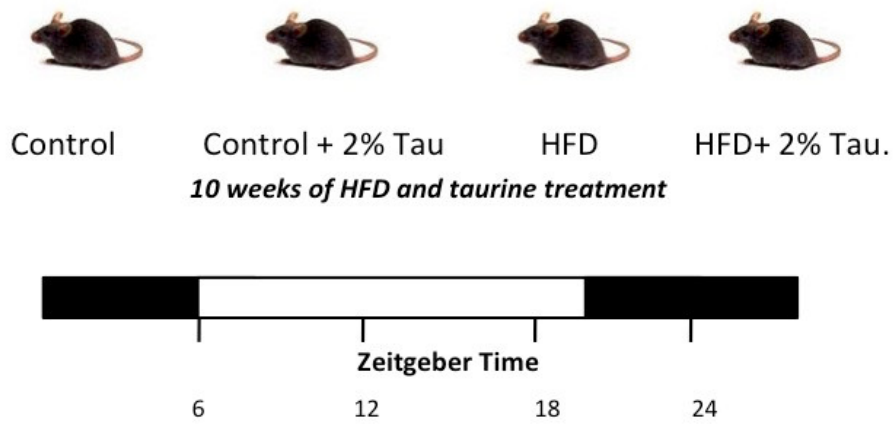
Blood samples were collected before the end of treatment to measure insulin and leptin during fed state. Plasma insulin and leptin were measured by ELISA (Mercodia Insulin, Uppsala Sweden and Crystal Chem, Harris County, TX, USA, respectively)..

3.1.4 Long-term treatment. For 10 weeks

We had the 4 experimental groups of each time point at 6:00, 12:00, 24:00 and 24:00. Throughout the study body weight, and water intake were measured every weekday during the10 weeks of diet in all groups. Food intake was monitored from the first week

of treatment by measuring the amount of food consumed from 8:00 to 20:00 (light cycle) and from 20:00 to 8:00 (dark cycle).

After 10 weeks of treatment, mice were euthanized at different times of day: 6:00, 12:00, 18:00 and 24:00h. GTT, ITT and insulinemia were measured in all groups as previously described. By the end of the 10 weeks the animals were euthanized. White adipose tissue was collected, weighted and stored for further gene expression analysis; furthermore, pancreatic islets were isolated to perform insulin secretion, gene expression and protein expression assays.



3.1.4.1 Circadian glucose, insulin and leptin.

To identify the daily pattern during fed state of glucose, insulin and leptin in all experimental groups were analysed at different times of the day 6:00, 12:00, 18:00 and 24:00. These parameters were measured at the end of the short term 1 week or long-term treatment of 10 weeks. After glucose measurements, blood samples were collected to measure insulin and leptin by ELISA. (Mercodia Insulin, Uppsala Sweden and Crystal Chem, Harris County, TX, USA, respectively).

At the end of 1 or 10 weeks of treatment animals were anesthetized at different times of the day according to each time point. Pancreatic islets were isolated and epididymal adipose tissue was removed, weighed, and divided for RNA extraction.

3.1.4.2 RNA extraction from adipose tissue.

Adipose tissue slices of 100 mg were placed in eppendorf tubes tube with 1 ml Trizol (Invitrogen). Samples were homogenized using zirconium oxide beads and the bullet blender homogenizer (Next Advance Inc). Homogenized samples were transferred to an eppendorf and centrifuged at 12000 rpm for 10 min at 4C. Supernatant was removed and samples were incubated at RT for 5 min. 200ul chloroform per 1 ml Trizol were added and samples were shacked vigorously for 15s and left for 5 min at RT, then samples were centrifuged at 12000G for 10 min at 4C. Aqueous phase (upper phase) was transferred to a new eppendorf. 500ul isopropyl alcohol per 1 ml Trizol was added gently mixed. Samples were incubated at 10 min at RT and centrifuge 12000G for 10 min at 4ºC. Supernatant was removed, pellets were washed with 1ml of 70% ethanol and centrifuged 7500 G for 5 min at 4ªC. Pellets were dried at RT for 1 hour and 30ul of RNAse free water were added.

RNA was quantified using a Nanodrop 1000 (Thermo Scientific Wilmington, MA) and then retrotranscribed using the High Capacity cDNA Reverse Transcription Kit (AB Applied Biosystems, USA) following the manufacturer's instructions. The cDNA was amplified by Real-Time PCR in a LightCycler 480 System (Roche) using Mesa Green qPCR Master Mix (Mesa Green, Eurogentec, Belgium). The expression of clock genes in adipose tissue was measured, with the housekeeping gene *36B4* (*ribosomal protein large P0*) used as the endogenous control for quantification. The results were expressed as the relative expression with respect to control levels ($2^{-\Delta\Delta ct}$).

3.1.4.3 Isolation of pancreatic islets

After being fully anesthetized, mice were euthanized by cervical dislocation; the abdominal cavity was open to allow the exposure of the pancreas and bile duct. After clamping the common bile duct as it joins the intestine, the pancreas was perfused with 2 ml of cold collagenase P solution (Sigma-Aldrich, St. Louis, MO, USA) diluted in Hanks balanced salt solution (Sigma-Aldrich). After inflation, the distended pancreas was removed and incubated at 37ºC for 6 minutes, islets of Langerhans were dispersed by gentle shaking. After digestion, islets were separated from exocrine tissue using a density gradient with Histopaque (Sigma-Aldrich, St. Louis, MO, USA) A previously described Casas et al 2008). The supernatant was discarded and the pellet resuspended in Hanks-BSA solution. Then, islets were rinsed into a Petri dish, isolated from any contaminating exocrine material and Islets were handpicked in a Leica stereomicroscope and frozen at -80 ºC for further measurements of gene and protein expression and for insulin secretion assays.

3.1.4.4 Glucose stimulated insulin secretion

After 10 weeks of treatment, mice were euthanized at 6:00, 12:00, 18:00 and 24:00. Groups of 5 fresh isolated islets from 4-5 different mice from each group were first pre-incubated at 37°C in a 5.6 mM glucose Krebs-Ringer bicarbonate buffer solution (KRBH) for 30 min. Supernatant was discarded, and islets were incubated for 60 min at 37°C in KRBH containing 2.8 mM or 16.7 mM glucose, respectively. After incubation, supernatants were collected, and insulin was quantified using a mouse insulin ELISA kit (Mercodia Insulin Uppsala, Sweden).

3.1.4.5 RNA isolation and Real-time PCR

Total RNA was prepared from isolated islets using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The concentration of RNA in the different samples was determined by measurement of the absorbance at 260 nm using a Nanodrop 1000 spectrophotometer (Thermo Scientific Wilmington, MA) and then retrotranscribed using the High Capacity cDNA Reverse Transcription Kit (AB Applied Biosystems, USA) following the manufacturer's instructions. The cDNA was amplified by Real-Time PCR in a LightCycler 480 System (Roche) using Mesa Green qPCR Master Mix (Mesa Green, Eurogentec, Belgium). The expression of clock genes in isolated pancreatic islets was measured, with the housekeeping gene *36B4* (*ribosomal protein large P0*) used as the endogenous control for quantification. The results were expressed as the relative expression with respect to control levels ($2^{-\Delta\Delta ct}$).

Primer sequences used for adipose tissue and pancreatic islets are shown in Table 4.

Table 4. Quantitative real PCR primers

Name	Sense primer (5 - 3)	Antisense primer (5 - 3)
Rev-erb alpha	GGTGCGCTTTGCATCGTT	GGTTGTGCGGCTCAGGAA
Clock	TTGCTCCACGGGAATCCTT	GGAGGGAAAGTGCTCTGTTGTAG
Bmal 1	GGACTTCGCCTCTACCTGTTCA	AACCATGTGCGAGTGCAGGCGC
Per 1	GCGGGTCTTCGGTTAAGGTT	AGGCTCAGCTGGGATTTGG
Per 2	ATGCTCGCCATCCACAAGA	GCGGAATCGAATGGGAGAAT
36B4	GAGGAATCAGATGAGGATATGGGA	AAGCAGGCTGACTTGGTTGC
Noc	TCATGCAGTGGAACATCCTC	TCAGGCACTTCCTCTCTTCC
Cdo	TCTGTGTGGCTGACGTTCTC	GAAGCTGCTGCAAGGAAATC

Csad	GGGCTCCATTACCAACTCAA	GACACCGGAGACAAAGTGGT
TauT	GATGACGGGTGAGGTGAAGT	TACGCATCCATCGTCATTGT
Pparγ	CACAATGCCATCAGGTTTGG	GCTGGTCGATATCACTGGAGATC
Aco	CCCAAGACCCAAGAGTTCATTC	TCACGGATAGGGACAACAAAGG
Cpt1α	CTCCTGAGCAGTTACCAATGC	GAACCTTGGCTGCGGTAAGAC
Hsl	AGAGACACCAGCCAACGGATA	TTTTGCGGTTAGAAGCCACA
C/ebpα	CTCTGGGATGGATCGATTGT	TTACAACAGGCCAGGTTTCC
C/ebpβ	AGCTGCTCCACCTTCTTCTG	CAAGCTGAGCGACGAGTACA
Glut4	ATTGGACGCTCTCTCTCCAA	GATTCTGCTGCCCTTCTGTC
Glut2	GGAAGAGGCATCGACTGAGCAG	GCCTTCTCCACAAGCAGCACAG
Gck	ATCTTCTGTTCCACGGAGAGG	TCTACAATGCCACGCTTCTG
Pdx1	GATGAAATCCACCAAAGCTC	TAAGAATTCCTTCTCCAGCTC
Ins2	TGGAGGCTCTCTACCTGGTG	TCTACAATGCCACGCTTCTG

3.1.4.6 Protein extraction

Protein extraction of isolated islets was obtained using RIPA lysis buffer (Tris 50 mmol/l, pH 7.5, EDTA 5 mmol/l, NaCl, 1% 150 mmol/l, Triton X-100 1%, SDS 0.1%, 10 mmol/l sodium fluoride, 1 % sodium deoxycholate, with 10% of protease inhibitor cocktail (Sigma) . For the extraction, islet lysates were frozen and thawed twice in 3 consecutive cycles of 2 minutes with different temperatures (-20ºC and 37ºC), followed by centrifugation for 20 min at 4°C. The supernatants were collected and transferred to a new microfuge tube. Protein concentration in lysates was determined with the Lowry protein assay kit (Bio-rad, Hercules, CA, USA), using following manufacturer's

instructions. Samples were stored at -20°C until required.

3.1.4.7 Western blot

Protein samples were resolved by 10% with SDS- PAGE (polyacrylamide gel electrophoresis). The gel was blotted for 1 h at 100 mV. The protein was electrotransferred onto a PVDF membrane (Perkin Elmer Life Sciences). Membranes were previously soaked in 100% methanol to hydrate them. The membranes were blocked for 1 h with 0.05% Tween-20 and 5% NFDM, and then incubated overnight at 4°C with the antibodies: AntiPer1 (1:500; Thermo Scientific Inc.) ß-Actin was used as a loading control (1:5,000; GE Healthcare, Hertfordshire, UK). Protein bands were revealed by using the Pierce ECL western blot substrate (Thermo Fisher Scientific, Madrid, Spain). The membrane was visualized with enhanced chemiluminescence on LAS. Respective bands were quantified by densitometry. Image J software 1.50a and intensity values for PER 1 were normalized with ß-Actin.

Buffers and solutions

Lysis Buffer
1ml Triton X-100%
10 mM sodium fluorite
10 mM sodium phosphate
50 mM Tris-HCl pH 7.5
5 mM EDTA
150 mM Nacl

Electrophoresis buffer 10x
25mM Tris
192 mM Glycine
1% SDS
Water
pH 8.3

TBS-Tween 0.05%
20mM Tris
150 mM NaCl
Tween-20 0.05%
Water

Laemmli 2X
0.5M Tris pH 6.8
40% Glycerol
SDS 10%
2- β mercaptoethanol 10%
0.004% Blue bromophenol

Transfer buffer 10x
25mM Tris
192 mM Glycine
pH 8.3

3.1.4.8 Whole islet Immunofluorescence and localization through immunofluorescence.

Fresh isolated islets were washed in buffer with PBS 1x and Triton 0.2% , then fixed in 4% of paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) for 20 min,. After this islets were permeabilized with PBS with Triton 0.3% and blocked in a PBS solution with Triton 0.5% and FBS 10% for 1h to minimizing unspecific binding of the primary antibody within the cell, and incubated overnight with the following primary antibodies: guinea pig anti-insulin 1:500, (Dako, Glostrup, Denmark) and anti-Per1 1:100 (Thermo Scientific, CA). Islets were incubated 2 hours with Secondary antibodies Cy3-labeled anti-guinea pig (Jackson Immuno research) and Alexa Fluor 488 (anti-rabbit (Molecular Probes, USA) were used at 1:200 dilutions, respectively. Nuclear staining was performed by using mounting media with DAPI (Life Technologies, USA).

Islets were mounted with an antifade mounting medium with Dapi (Thermo Fisher Scientific, MA, USA), in a μ chamber-slide with coverslip-like bottom (IBIDI, WI, USA). Immunofluorescence was assessed in a confocal laser microscope (Leica. Microsystems, Wetzlar Germany). For each individual islet images were acquired every 10μm using a 40x oil immersion objective.

The same confocal settings (i.e pinhole, smart gain, smart offset, phase, zoom) were maintained for each islet in all groups. Images were analyzed by Image J Software 1.50a (NIH, USA). Analysis was done by counting the number of stained positive cells represented by percentage, using imageJ 1.50d software (NIH, USA)

3.1.5 Statistical analysis

Values are presented as means ± SEM. Differences between two groups were analyzed by Student's *t* test. The effect of time and groups differences were measured by one-way or two-way ANOVA, with Bonferroni post hoc test for multiple comparisons, where appropriate. All statistical tests were performed with a level of significance $P > 0.05$. In orden to perform a circadian analysis in different parameters, cosinor analysis was done using the Acrophase program (R. Refinetti 2004) for fitting cosine functions to the data using a fixed 24 hour period and included the mesor (middle value of the fitted cosine representing a rhythm-adjusted mean), the amplitude (difference between the minimum and maximum of the fitted cosine function), the acrophase (the time at which the peak of a rhythm occurs, expressed in hours) and fitted cosine values to calculate the goodness of the fit by coefficient of determination $R^{z.}$

4. RESULTS

4.1 RESULTS

4.1.1 Short- term treatment of 1 week of HFD and taurine.

To check the effect of taurine at the beginning of the treatment we next measured body weight, visceral fat, water and food intake at the first week of treatment. There was no difference in body weight, visceral fat and water intake in all experimental groups (Fig.8A-C, respectively).

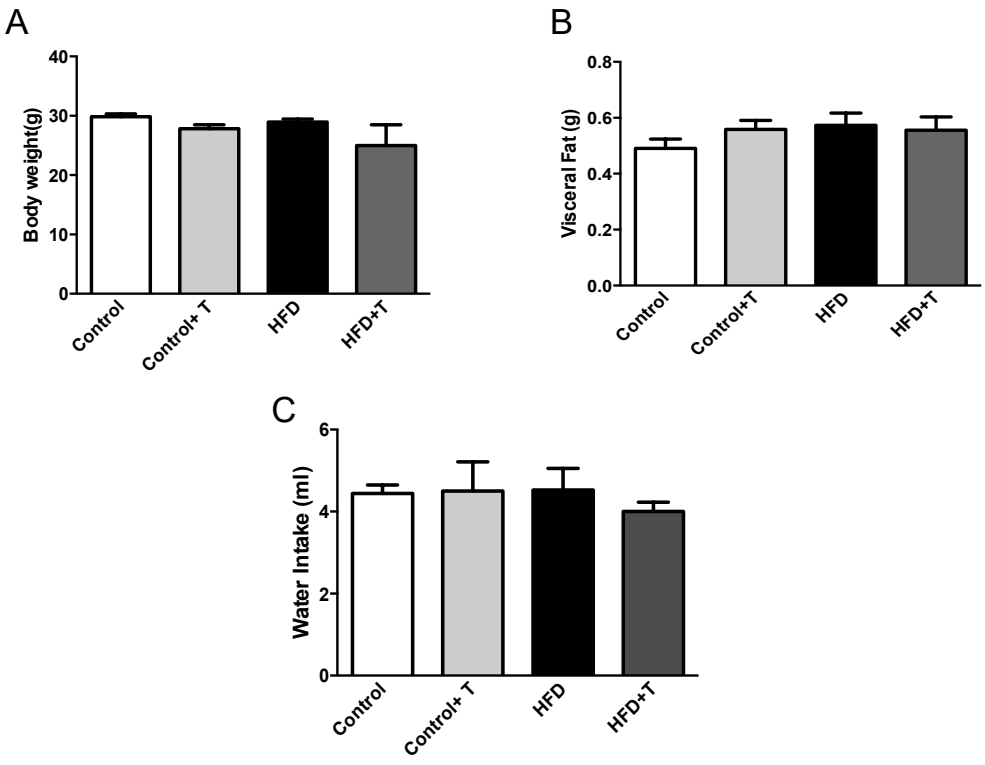


Figure 8. Body weight, visceral fat, water intake and food intake of 1 week of HFD and taurine treatment. Data are expressed as mean ± SEM. (** $p<0.01$ *** $p<0.001$).

4.1.1.1 Effects of taurine on food intake and insulin.

Mice fed a HFD had an increase in food intake during the light and dark cycles already at the first week of treatment whereas taurine was able to prevent the increase in food intake caused by HFD in both light and dark cycles (Figure 9A). Showing that one-

week of taurine treatment in mice treated with HFD can decrease food intake during light and dark cycles even before any change in body weight and visceral fat. The increase in food intake was followed by an increase in plasma insulin (Figure 9B) during day and a strong tendency at night-time. Plasma leptin levels were significantly higher in HFD groups, however taurine was not able to decrease leptin already at one-week of treatment.

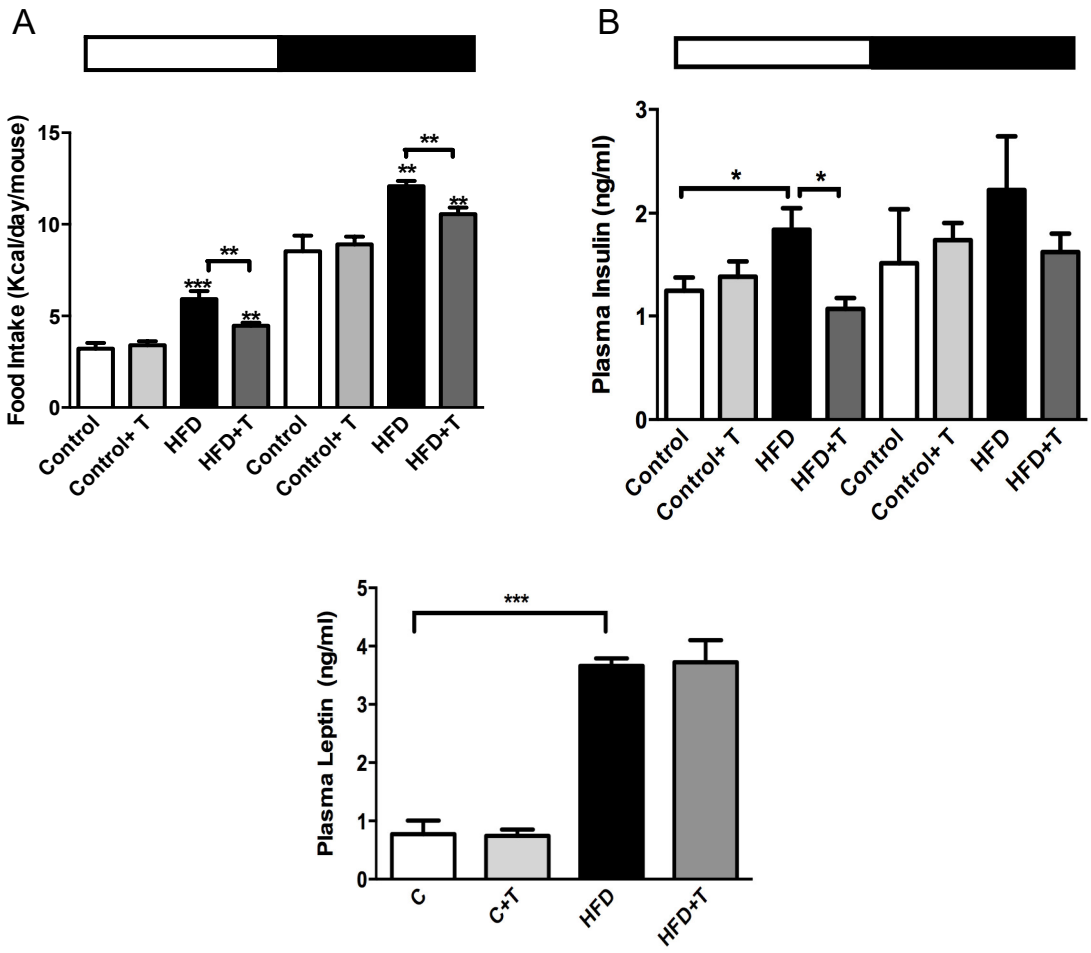


Figure 9. Food intake and insulin during day and night of 1 week of HFD and taurine treatment.

Plasma leptin levels after 1 week of intervention. Data are expressed as mean ± SEM. (* p<0.05 , ** p<0.01 *** p<0.001).

4.1.1.2 Daily glucose and insulin at 1 week of treatment.

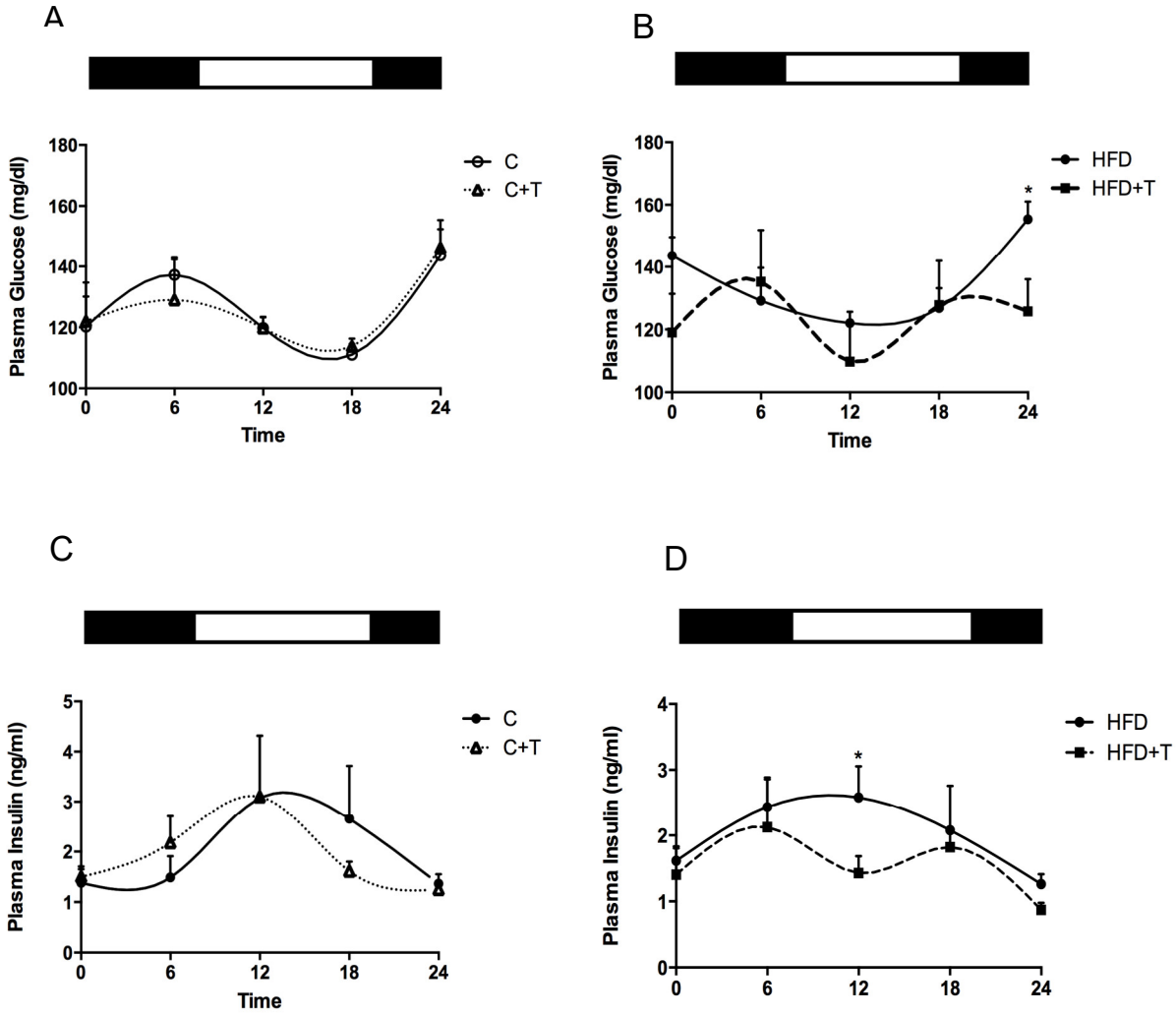


Figure 10. Effects of taurine treatment on daily glucose and insulin

(A) 24h time course measurements of plasma glucose concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+ T ($^{\&}$ P< 0.05). Differences between C vs HFD (* P<0.05, **P<0.001). Differences between HFD vs HFD+ T ($^{\#}$ P < 0.05, $^{\#\#\#}$ P< 0.001). (n= 8-10 mice per group). **(B)** 24h time course measurements of plasma insulin concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+ T ($^{\&}$ P< 0.05). Differences between C vs HFD (*** P<0.001). Differences between HFD vs HFD+ T ($^{\#}$ P< 0.05, $^{\#\#}$ P< 0.01) (n=8-10 mice per group).

4.1.1.3 Glucose tolerance and insulin sensitivity

One week of HFD treatment led to glucose intolerance (Fig.11A) and decreased insulin sensitivity (Figure 11C) but taurine treatment in mice fed a HFD had a small effect on glucose tolerance and insulin sensitivity with no statistically significant results when calculating the area under the curve (Figure 11B and 11D respectively).

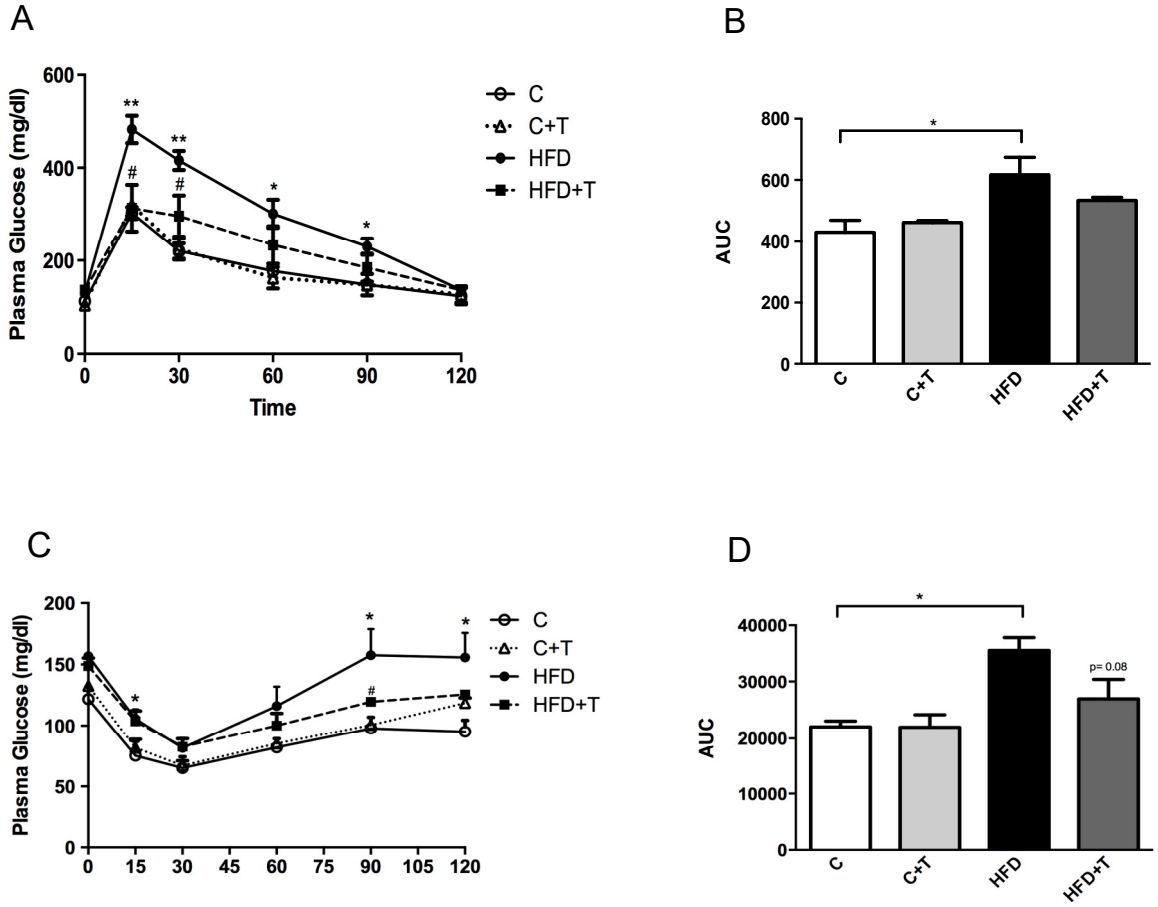


Figure 11. Effects of taurine treatment on glucose tolerance and insulin sensitivity after 1 week of treatment.

Glucose tolerance test (A). Differences between C vs HFD (*P <0.05, ** P <0.01); HFD vs HFD+ T ($^{\#}$ P < 0.05). (n=5-7 mice per group). (B) Area under the curve (AUC). (C) Insulin tolerance test. Differences between C vs HFD (* P <0.05); HFD vs HFD+ T ($^{\#}$ P < 0.05). (n=5-7 mice per group) (D) Area under the curve (AUC). Data are expressed as mean ± SEM.

4.1.1.4 Clock genes expression at 1 week

We next checked whether taurine could modulate the expression of *clock genes* in isolated pancreatic islets at one week of treatment. The expression of *Clock, Bmal1, Rev-erb α and Per1* was similar in all groups. Showing that there were no effect of HFD on clock gene expression, whereas taurine did not induce changes.

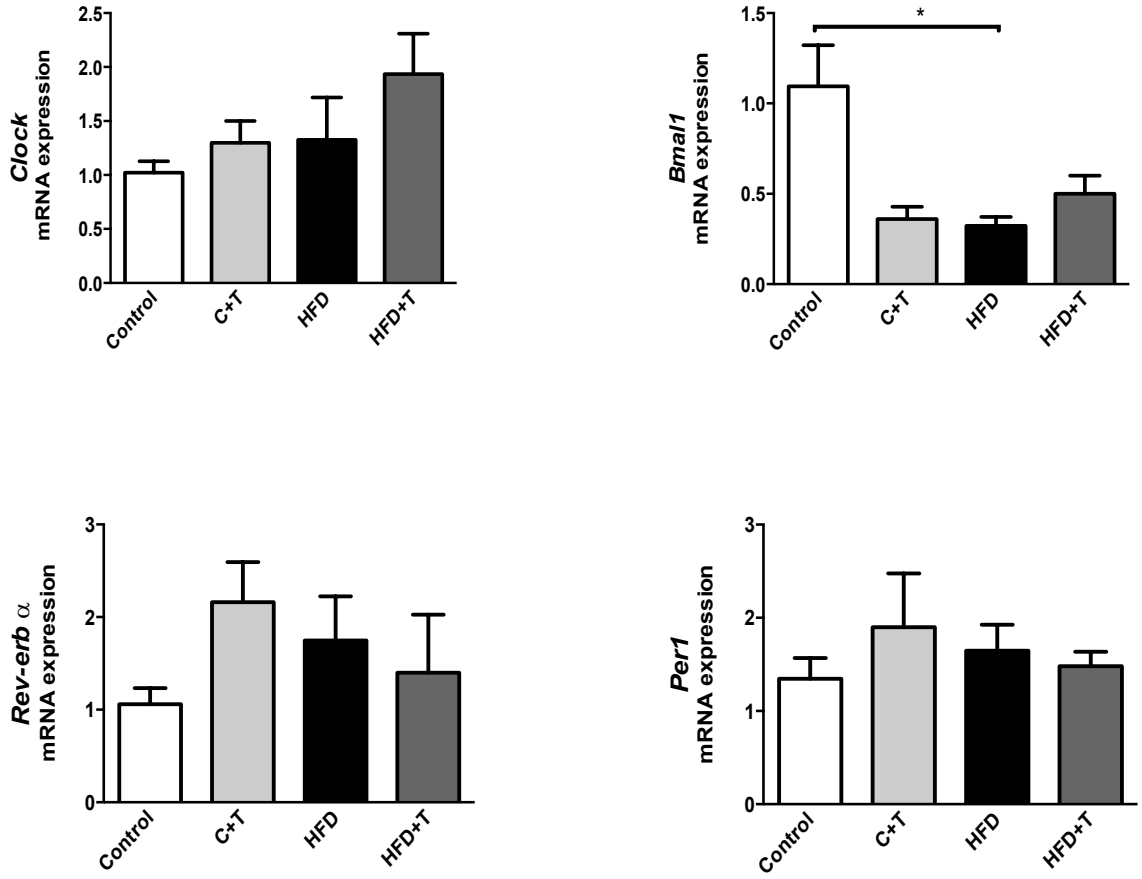


Figure 12. Expression of the clock genes *Clock, Bmal1, Rev-erb α, Per1*, in pancreatic islets at 1 week of HFD and taurine treatment.
Data are expressed as mean ± SEM. Differences between C vs HFD * P<0.05.

4.1.2 Long- term treatment of 10 weeks

4.1.2.1 Effects of taurine treatment on body weight and visceral fat

We first measured body weight progression from the first week of treatment. Body weight was similar between the groups until the 5th week of treatment. In the HFD group, body weight increased from the 5th week of treatment in both HFD and HFD+T treated mice, as compared to controls. However, from the 8th to the 10th week of treatment, mice fed with HFD+T prevented the increase in body weight, compared to mice fed with HFD until the end of treatment (Figure 13A). After 10 weeks of treatment, visceral fat weight was comparable between the C and C+T groups. As expected, mice treated with a HFD showed a significant increase in visceral fat, compared to the C group, whereas mice treated with HFD+T had a decrease in visceral fat, compared to the HFD group (Figure 13B).

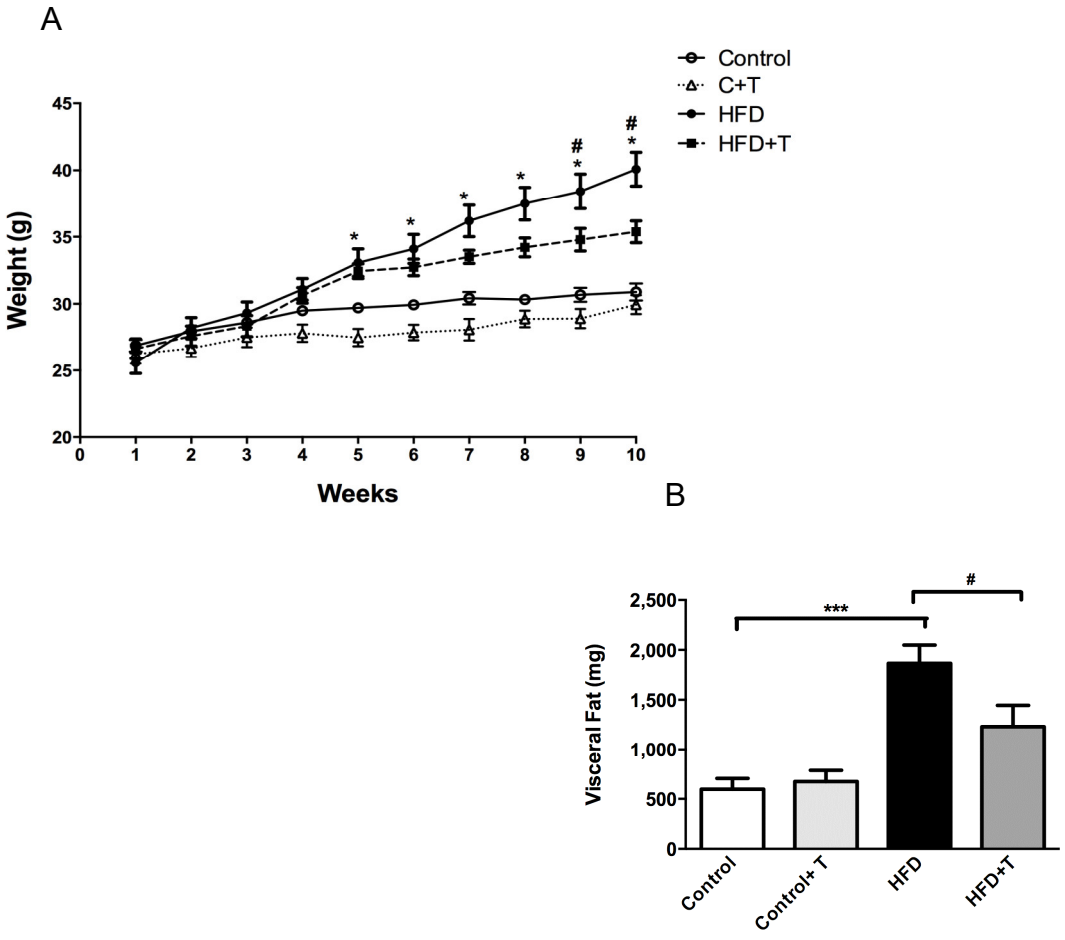
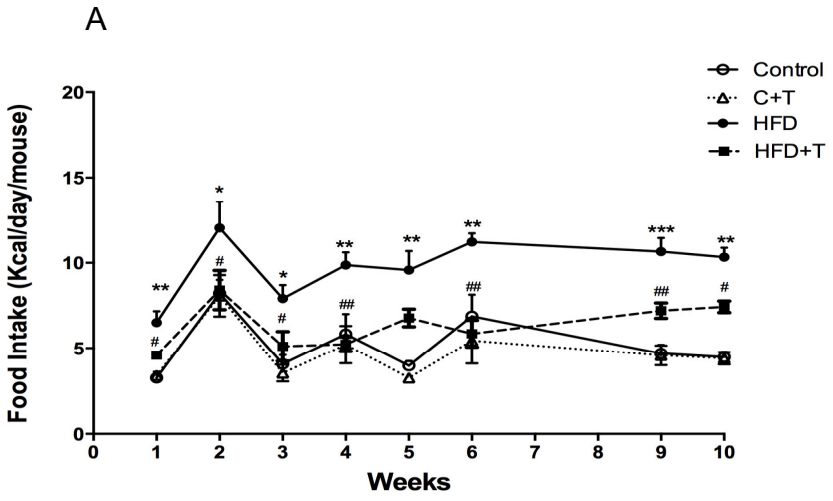


Figure 13. Effects of taurine treatment on body weight, visceral fat, food intake. Mice were treated with chow diet (C), Chow+ Taurine (C+T), High-fat diet (HFD) or HFD+ Taurine (HFD+T) for 10 weeks. **(A)** Body weight progression from the first day of taurine treatment until the 10th week of treatment in mice fed with chow or HFD. (n=8-10 mice per group). Differences between C vs HFD (* P<0.05); HFD vs HFD+ T (# P< 0.05, ## P< 0.01). **(B)** Visceral fat weight after 10 weeks of taurine treatment in mice fed with chow or HFD. Differences between C vs HFD (* P<0.05); HFD vs HFD+ T (# P< 0.05). (n=8-10 mice per group).

4.1.2.2 Effects of taurine on food and water intake.

Measurements of food intake Interestingly, HFD-treated mice had increased food intake already at the first week of treatment with a peak of food consumption at the second week and a sustained elevation of food intake until the end of treatment. On the other hand, HFD+T mice had decreased food intake already at the first week compared to the HFD group (Fig.14A). At the end of treatment (10th week) during the light cycle was similar between C and C+T groups and increased during the dark cycle in both groups (p 0.0001, respectively). In contrast, mice fed a HFD exhibited an increase in food intake during both light and dark cycles, as compared to C group (Figure 14B), Taurine decreased food intake in both light and dark cycles. Water intake was similar in all experimental groups at the 10th week of treatment (Figure 14C).



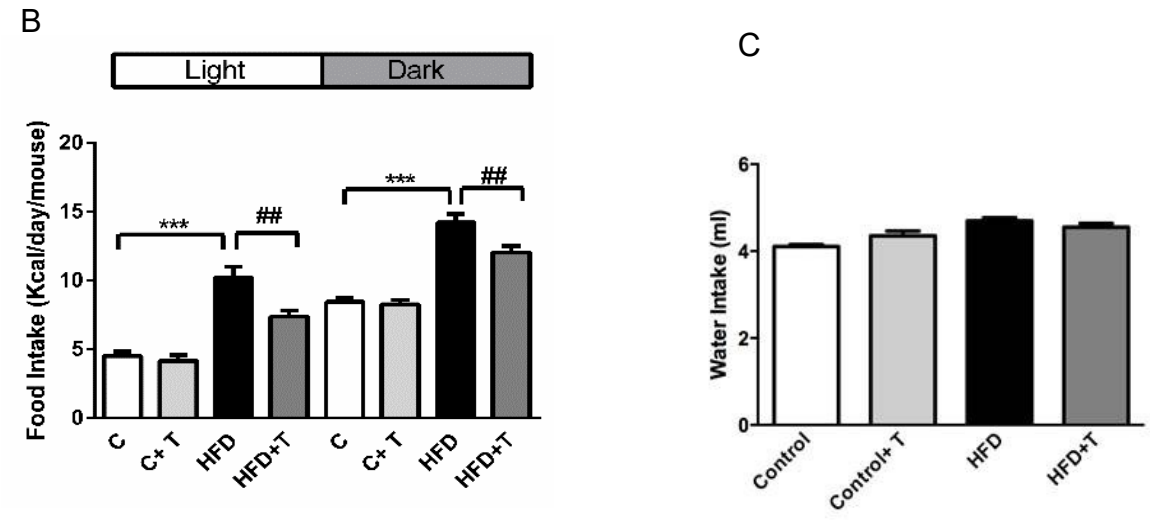


Figure 14. Food intake and water intake after 10 weeks of intervention.

(A) Food intake progression from the first week until the 10th week of treatment. Differences between C vs HFD * P<0.05. ** P<0.001, ***P<0.0001. Differences between HFD vs HFD+T (#P<0.05, ## P<0.001). **(B)** Food intake at 10th week of treatment during the light and dark cycle. Food was measured by weighing the food consumption separately during the day (from 8:00 to 20:00) and the food consumption during the night (from 20:00 to 8:00). Differences between C vs HFD (*** P<0.001); and HFD vs HFD+ T (## P< 0.01). **(C)** Water Intake at 10th week of treatment. Data are expressed as mean ± SEM.

Table 5. Effects of taurine treatment on body weight,visceral fat and food intake.

Variable	Groups						
	C	C+T	HFD	HFD + T		P Value	
Body weight gain (g)	4 ± 0,8	3,7 ± 0,5	14,5 ± 0,9 ***	8,7 ± 0,8 ##	0,76	0,001	0,01
Visceral fat (mg)	596,4 ± 109,7	673,5 ± 112,6	1866,3 ± 183,5 ***	1229,8 ± 214,7#	0,63	0,001	0,03
Food intake (kcal/day/mouse)	6,4 ± 0,3	5,9± 0,3	11,05 ± 0,4 ***	8,5 ± 0,4 #	0,32	0, 001	0,05

C vs HFD * p<0.05 HFD vs HFD+T # p<0.05
** p<0.01 ## p<0.01
*** p<0.001 ### p<0.001

Shown are the means ± SEM

4.1.2.3 Effects of taurine on daily glucose, insulin and leptin levels at 10th weeks treatment.

The effect of taurine treatment (C+T group) on the daily glucose levels was evident at time 12:00, with lower levels than in the C group (Figure 15A). As expected, blood glucose levels were elevated at 18:00 and 24:00 in the HFD group, compared to the C group (Figure 15A). However, mice treated with HFD+T exhibited lower glucose levels throughout the 24h period as compared to mice treated only with HFD (Figure 15A). Mesor analysis of the data confirmed the hypoglycemic effect of taurine during HFD treatment by decreasing glucose levels to the same levels of the control group (Table 5). Plasma insulin levels were similar in the C group at all time points measured, but increased in the C+T group at 24:00 (Figure 15B). In contrast, insulin levels were continuously elevated throughout the 24h period in the HFD group as compared to the C group. Interestingly, HFD+T decreased the overall levels of plasma insulin throughout the 24h period and increased plasma insulin levels at 24:00 (Figure 15B).
Control mice exhibited statistically significant variations in plasma leptin concentrations with decreased values at 12:00, and a peak at 24:00 ($P<0.01$ and $P<0.05$). The C+T group exhibited the same variations in plasma leptin, but the peak occurred at 18:00 (Figure 15C). Interestingly, HFD mice disrupted the daily pattern of leptin, with no decrease in leptin levels at 12:00, a peak of leptin at 18:00 ($P<0.05$). Taurine treatment prevented the disruption of daily plasma leptin caused by HFD, decreased leptin levels at 12:00 ($P<0.01$) and a peak of leptin from 12:00 to 24:00 ($P<0.01$). Confirming these results, the mesor and amplitude values showed that taurine decreased insulin and leptin levels in mice treated with a HFD (Table 5). Our results demonstrated that taurine supplementation in mice fed a HFD can restore the 24h pattern of plasma leptin levels.

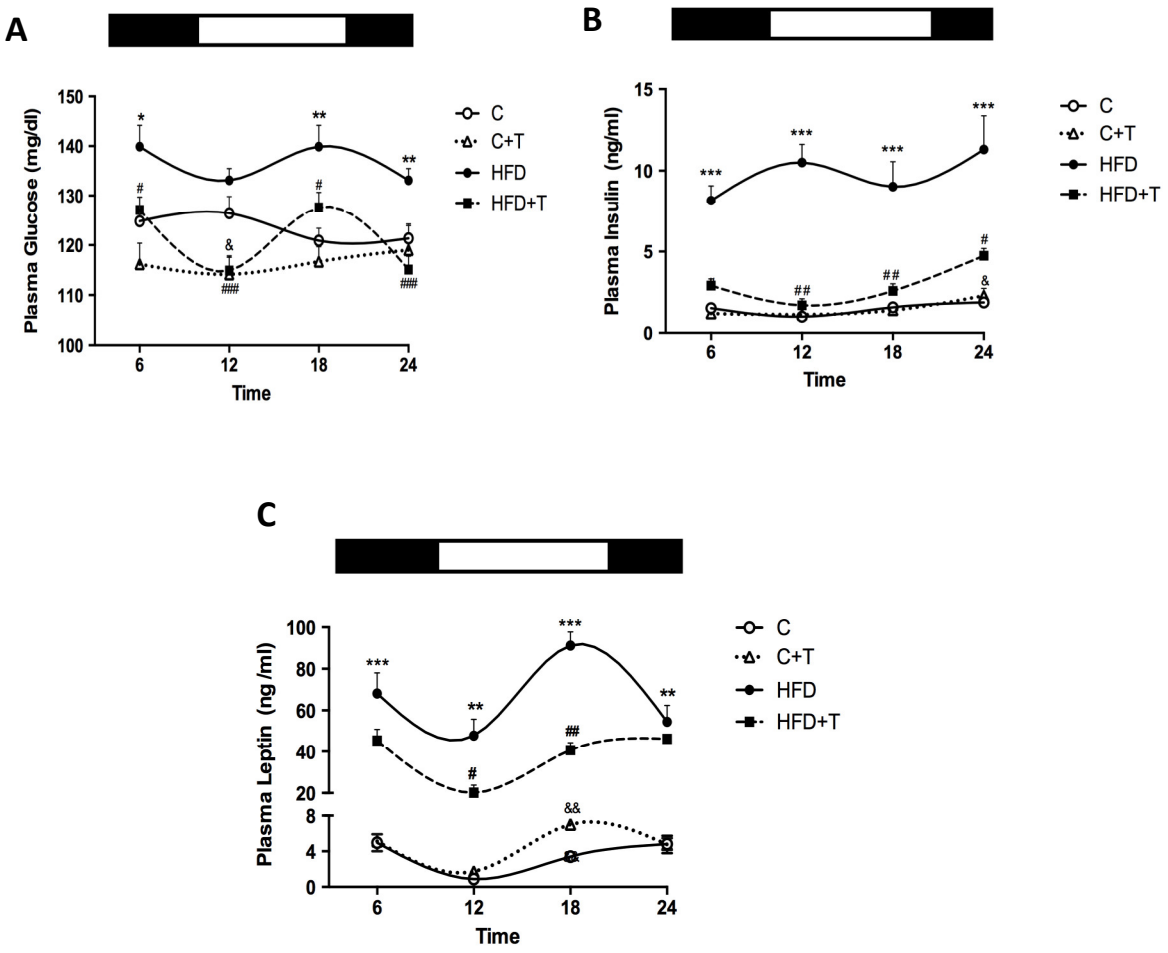
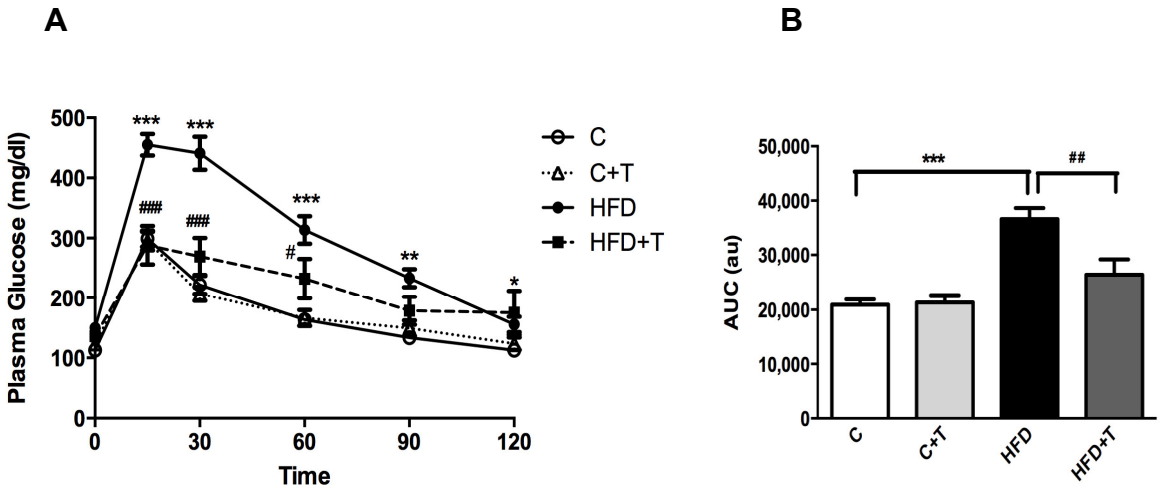


Figure 15. Effects of taurine treatment on daily glucose, insulin and leptin levels.

(A) 24h time course measurements of plasma glucose concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+ T ($^{\&}$ P< 0.05). Differences between C vs HFD (* P<0.05, **P<0.001). Differences between HFD vs HFD+ T ($^{\#}$P < 0.05, $^{\#\#\#}$ P< 0.001). (n= 8-10 mice per group). **(B)** 24h time course measurements of plasma insulin concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+ T ($^{\&}$ P< 0.05). Differences between C vs HFD (*** P<0.001). Differences between HFD vs HFD+ T ($^{\#}$ P< 0.05, $^{\#\#}$ P< 0.01) (n=8-10 mice per group). **(C)** 24h time course measurements of plasma leptin concentrations after 10 weeks of taurine
(HFD+T). Differences between C vs C+T ($^{\&\&}$ P< 0.01). Differences between C vs HFD (** P<0.05, *** P<0.001). Differences between HFD vs HFD+T ($^{\#}$ P< 0.05, $^{\#\#}$P < 0.01). (n=8-10 mice per group). The black bars refers on the top of the figures to the dark cycle and the white bars to the light cycle.

4.1.2.4 Effects of taurine on glucose tolerance and insulin sensitivity

Glucose tolerance was similar between C and C+T mice, showing no differences between the groups. As expected, HFD mice displayed impaired glucose tolerance, evident in the total area under the curve (AUC) as compared to control mice (Figure 16A and B). Taurine supplementation in HFD-fed mice prevented glucose intolerance caused by a HFD, as indicated in the total area under the curve (AUC) (Figure 16B). Plasma insulin levels during the ipGTT showed a significant decrease in plasma insulin levels in the C+T group at 15 min. In accordance with the ipGTT results, the HFD group displayed elevated insulin levels at 30 and 60 min, as compared to the C group. Interestingly, taurine treatment reduced insulin levels at 30 min and 60 min in animals treated with HFD (Figure 16C), as indicated in the total area under the curve (AUC) (Figure 16D). There were no differences in insulin sensitivity between C and C+T mice, but the HFD group exhibited impaired insulin sensitivity (Figure 16E). Ten weeks of taurine treatment were sufficient to prevent insulin resistance caused by HFD feeding, as shown in the total area under the curve (AUC) (Figure 16E and 16F).



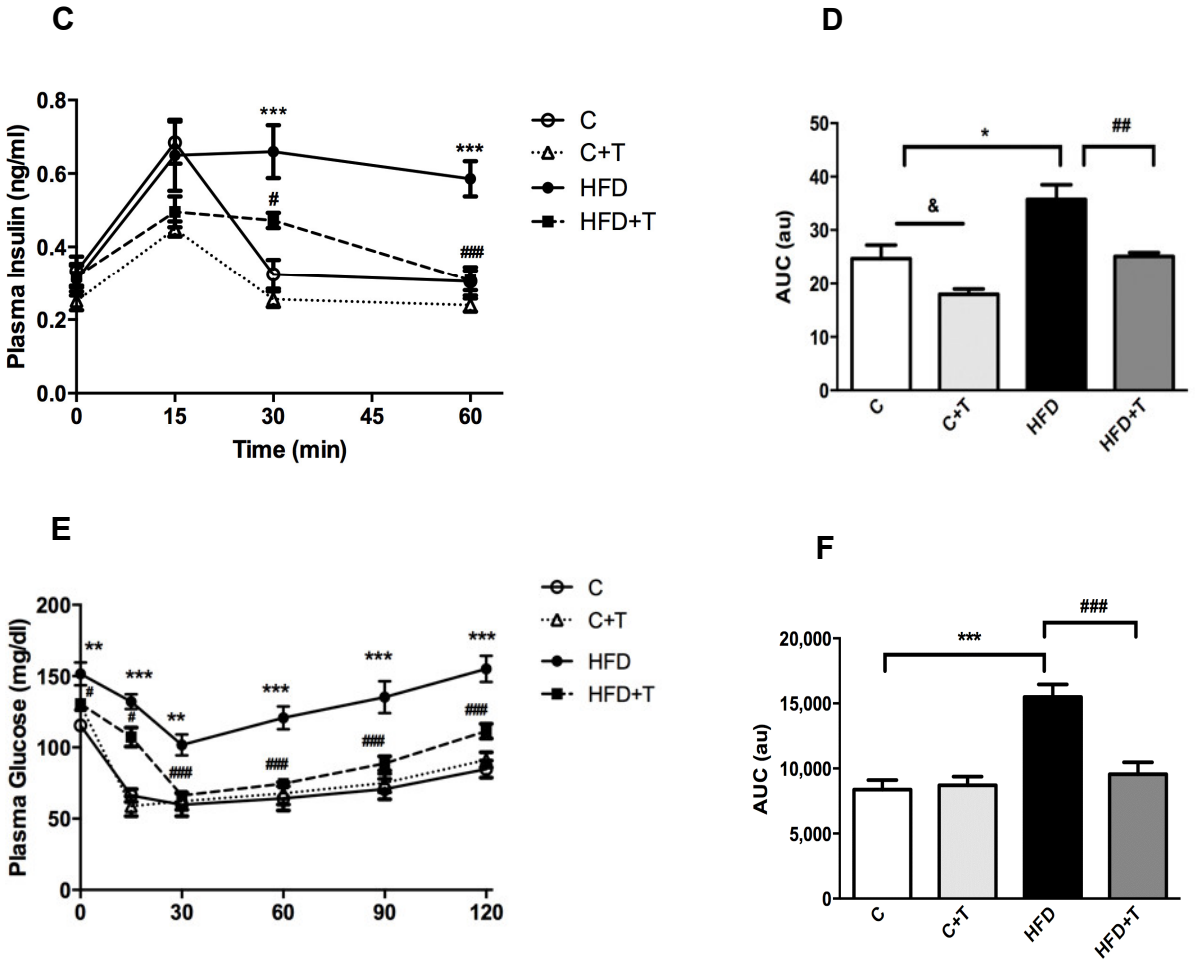


Figure 16. Effects of taurine treatment on glucose tolerance and insulin sensitivity.

Glucose tolerance test **(A)** (C), (C+T), (HFD) and (HFD+T). Differences between C vs HFD (* P <0.05, ** P <0.01*** P <0.001); HFD vs HFD+ T ($^{\#}$ P < 0.05, $^{\#\#\#}$ P < 0.001). (n=8-10 mice per group) **(B)** Area under the curve (AUC). Differences between C vs HFD (*** P <0.001) and HFD vs HFD+T (** P <0.01). **(C)** Insulin tolerance test (n=8-10). Differences between C vs HFD (** P <0.01*** P<0.001); HFD vs HFD+ T ($^{\#}$ P < 0.05, $^{\#\#\#}$ P< 0.001). **(D)** Area under the curve (AUC), (*** P<0.001). **(E)** Plasma insulin measured during the glucose tolerance test at 0, 15, 30, and 60 min (n=5). Differences between C vs HFD (*** P<0.001); HFD vs HFD+ T ($^{\#}$ P< 0.05, $^{\#\#\#}$ P< 0.001) and C vs C+T ($^{\&\&}$ P< 0.01). (n=8-10 mice per group). **(F)** Area under the curve (AUC) ($^{*,\&}$ P<0.05) ($^{\#\#}$ P < 0.01).

4.1.2.5 Effects of taurine on daily insulin secretion in vitro

To check whether the modulation of taurine on the 24h pattern of insulin secretion in vivo reflects changes in insulin secretion in vitro, we next performed glucose-stimulated insulin secretion from fresh isolated islets at 6:00, 12:00, 18:00, and 24:00. Isolated islets from control mice exhibited no changes in insulin secretion during the 24h period at low glucose concentrations. Islets from the C+T group had increased insulin secretion at 18:00 when stimulated with basal glucose concentrations (Figure 17A). When control islets were stimulated with high glucose concentrations, we could observe the effect of the time of the day on insulin secretion. Glucose-stimulated insulin secretion had a peak of secretion at 18:00 and maintained high levels at 24:00 as compared to secretion at 6:00 and 12:00. The same pattern of glucose-stimulated insulin secretion was observed in islets from the C+T group, however, taurine treatment augmented glucose-stimulated insulin secretion during the dark cycle, as compared to secretion from control islets (Figure 17A). When islets from HFD mice were stimulated with high glucose concentrations, we found alterations in insulin secretion according to the time of day. Islets isolated at 6:00 lost the stimulatory effect of glucose compared to control islets (Figure 17B). The nocturnal increase of glucose-stimulated insulin secretion was advanced to 12:00, exhibiting an increase in insulin secretion already at 12:00 and keeping high levels throughout the dark cycle. We could not detect the effect of the time of day in isolated islets from mice treated with HFD+T on glucose-stimulated insulin secretion. However, islets from HFD+T decreased glucose-induced insulin secretion at 12:00, 18:00 and 24:00, as compared to the HFD group (Figure 17B). These results indicate that HFD disrupts in vitro glucose-stimulated insulin secretion that could not be prevented by taurine treatment.

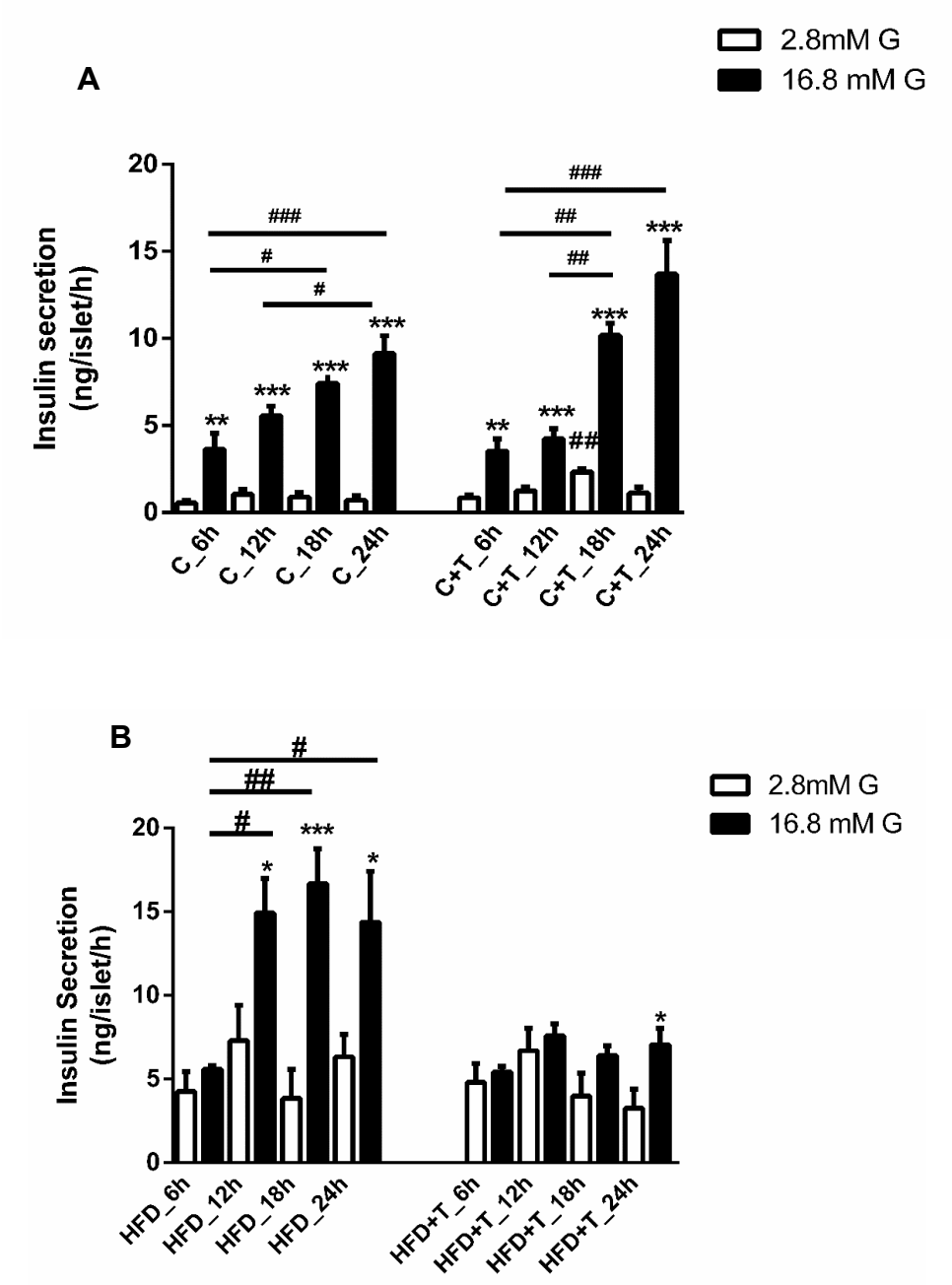


Figure 17. Effects of taurine on circadian insulin secretion in vitro

Pancreatic islets were isolated at different times of day (6:00h, 12:00h, 18:00h and 24:00h) after 10 weeks of taurine treatment and stimulated with 2.8mM glucose (white bars) and 16.8 mM glucose (black bars) (n=6-7 mice per group). **(A)** Insulin secretion from C and C+T groups. Data are expressed as mean ± SEM. Differences between 2.8mM glucose and 16.8 mM glucose in the C (**P <0.01, ***P <0.001) and C+T groups (**P <0.01, ***P <0.001). Differences in the time of day at 2.8mM glucose and 16.8 mM glucose in the C and C+T groups ($^{\#}$P<0.05, $^{\#\#}$ P<0.01, and $^{\#\#\#}$ P<0.001 respectively). **(B)** Insulin secretion from HFD and HFD+T groups (n=6-7). Differences between 2.8mM glucose and 16.8 mM glucose in the HFD group (*P<0.05, ***P<0.001) and HFD+T group (*P<0.05). Differences in the time of day at 16.8 mM glucose in HFD group ($^{\#}$P<0.05, $^{\#\#\#}$ P<0.001).

4.1.2.6 Expression of *Cdo, Csad* and *Taurine transporter* in pancreatic islets.

To analyse if decreased levels of rate-limiting enzyme for taurine biosynthesis, cysteine dioxygenase (*Cdo*), cysteine-sulfinic acid decarboxylase (*Csad*) and the taurine transporter (*TauT*) are affected in obesity we examine the levels of these genes in pancreatic islets [158,236]. Taurine is accumulated in various tissues, including pancreas [237]. In our results *Cdo*, *Csad*, important enzymes of taurine biosynthesis and the taurine transporter (*TauT*) are well expressed in pancreatic islets. *Cdo, Csad* and *TauT* mRNA levels were decreased in HFD group and taurine restored their levels (Figure 18A,B). These results suggest that a HFD alters the expression of these parameters, which are key factors in determining taurine flux.

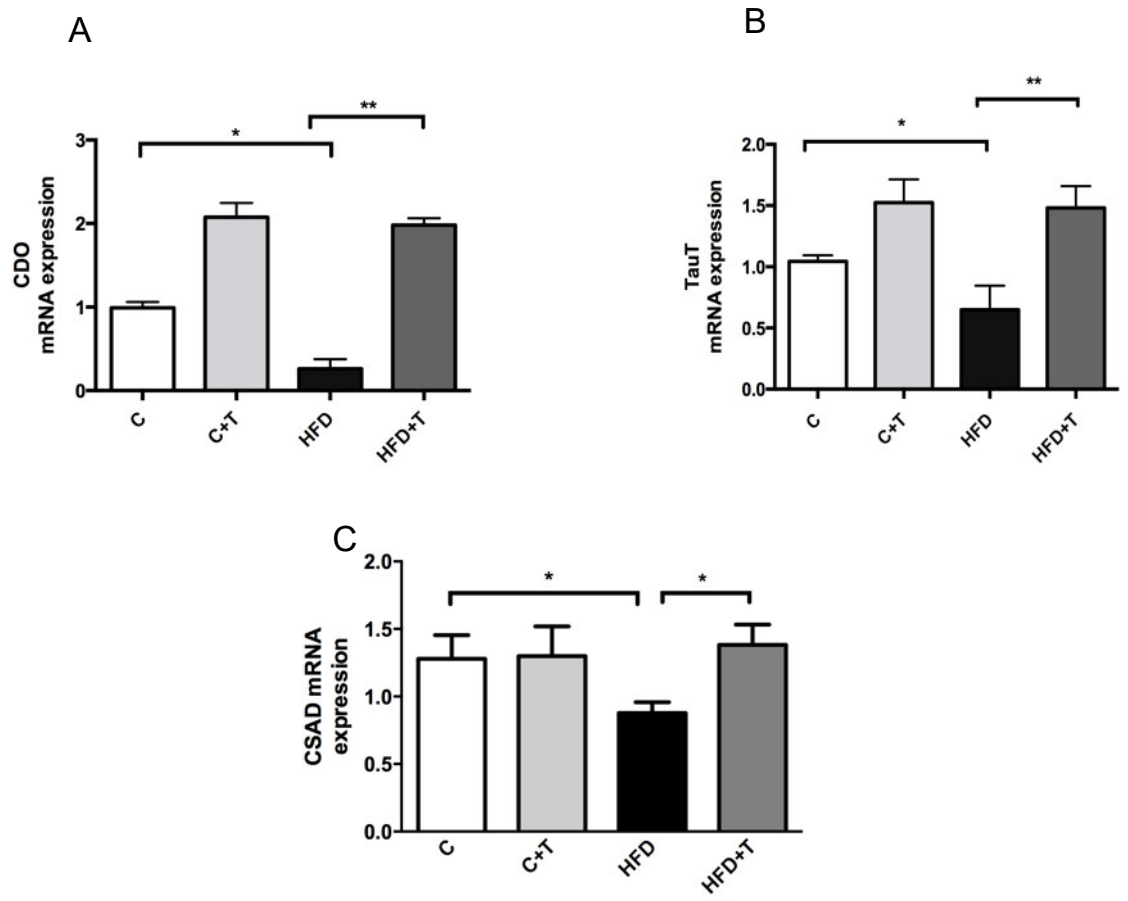


Figure 18. Cdo, Csad and TauT expression in pancreatic islets after 10 weeks.
Data are expressed as mean ± SEM. Differences between (* P<0.05 **P<0.01)

4.1.2.7 Expression of genes involved in pancreatic islet function.

We measured the expression of genes involved in islet function. There were no differences between groups in the Glucose transporter (*Glut2*) and Glucokinase (*Gck*). In contrast, HFD islets presented upregulation of duodenal home box factor (*Pdx1*) but its expression was not modify by taurine. In addition HFD displayed increased levels of Ins2 than control, whereas taurine did not change Ins2 expression levels. On the other hand, *Pparγ* been shown to regulate directly key β cell genes involved in glucose sensing and sensitivity. HFD decreased the expression of pancreatic islets when compared with control, in contrast taurine normalizes the expression of *Pparγ*.

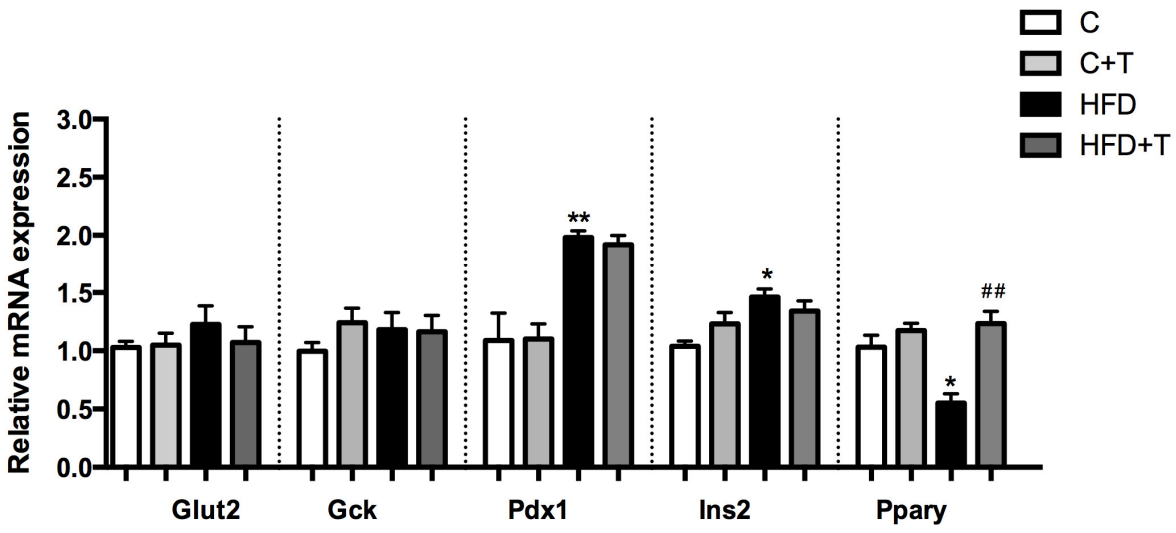


Figure 19. Gene expression of *Glut2, Gck, Pdx1, Ins2* and *Pparγ* in pancreatic islets.
Data are expressed as mean ± SEM. Differences between C vs HFD (*p<0.05, **p<0.01). HFD vs HFD+T ($^{\#}$ p<0.05, $^{\#\#}$ p<0.01)

4.1.2.8 Effects of taurine on Nocturnin, a clock controlled gene in pancreatic islets.

Nocturnin is a recently studied enzyme; a circadian deadenylase expressed at high levels during the night in liver and adipose tissue and has been implicated in obesity [66,238]. Nocturnin is considered to be a clock-controlled gene (CCG). We checked the mRNA levels of Nocturnin through 24h in pancreatic islets, showing that is well expressed in islets with a peak at the beginning of the night in control mice. HFD mice displayed increased levels compared to control with the highest peak at at 18h and 24h. Interestingly taurine changed the expression of Noc by decreasing its levels at the end of the light cycle (Figure 20). These results show that taurine could be modifying Nocturnin expression in pancreatic islets during obesity and diabetes.

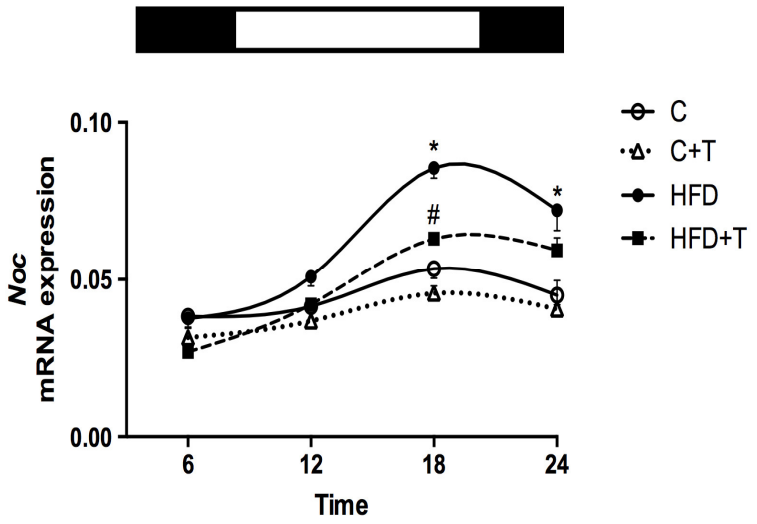
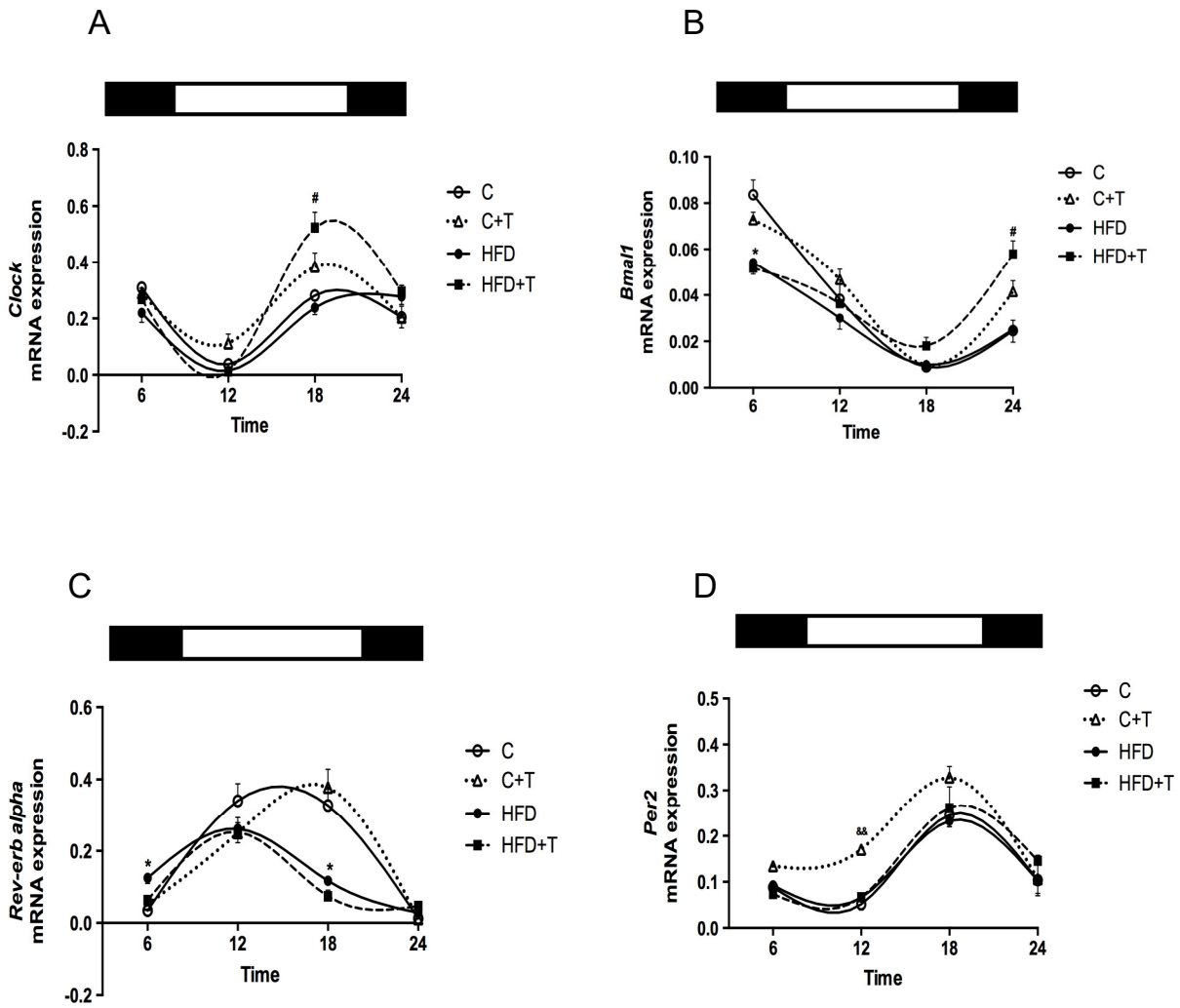


Figure 20. Nocturnin expression in pancreatic islets during 24h.
Ccrn4l gene expression in isolated islets. ⊖ (C), ·△· (C+T), ●— (HFD) and ■· (HFD+T). Data are expressed as mean ± SEM. Differences between (*,# P<0.05)

4.1.2.9 Effects of taurine on the clock gene expression in pancreatic islets.

The islet exhibits oscillations in clock gene expression throughout the day and alterations in clock gene expression impaired beta-cell function leading to the development of diabetes [191,227,228]. Therefore, we next checked whether taurine could modulate the daily pattern of *Bmal1*, *Clock*, *Rev-erbα*, *Per1* and *Per2* expression in isolated pancreatic islets. The expression of *Clock* was similar in control or C+T islets (Figure 21A). There was no effect of HFD on *Clock* expression, but taurine treatment in the HFD group increased *Clock* expression at 18:00 (Figure 21A). This effect was evident by the mesor and amplitude values showing an increase in the HFD+T group (Table 7). The 24h pattern of *Bmal1* in control islets showed the highest peak at 6:00 and the lowest at 18:00 (Figure 21B). Taurine treatment in mice fed with chow diet showed an increase in mesor values detected by the cosinor analysis. Islets from HFD downregulated *Bmal1* expression at 6:00, as compared to control islets, whereas HFD+T islets exhibited increased *Bmal1* expression at 24:00 (Figure 21B). Cosinor analysis showed that HFD feeding decreased the mesor and amplitude of *Bmal1* expression and that taurine treatment restored mesor values of *Bmal1* expression and changed the acrophase (from 6 in all groups to 23h) in mice treated with HFD (Table 7). *Rev-erbα* expression showed a similar pattern of expression in C and C+T islets, with a peak of expression at 12:00 and 18:00 and a decrease at 6:00 and 24:00 (Figure 21 C). HFD disrupted 24h expression by upregulating *Rev-erbα* expression at 6:00 and downregulating at 18:00, compared to C islets, while there was no effect of taurine in HFD+ T islets on *Rev-erbα* expression (Figure 21C). Cosinor analysis confirmed this data, showing a decrease in the values of the mesor in the HFD group, in relation to the C group (Table 7). *Per2* expression had a peak of expression at 18:00 in all experimental groups, C+T increased *Per2* levels at 12:00 compared to control. There were no changes in HFD groups (Figure 21D). In C and C+T islets, *Per1*

showed changes during the 24h pattern of expression with the highest peak at 18:00 (Figure 21E). HFD downregulated *Per1* expression at 6:00 and at 18:00, as compared to C islets. Interestingly, taurine treatment during HFD feeding restored the circadian pattern of *Per1* expression to the same expression levels as in the C islets (Figure 21E). Cosinor analysis confirmed the inhibitory effect of HFD on *Per1* expression and in the amplitude of the gene, as well as the preventive effect of taurine on the *Per 1* expression and *Per1* amplitude (Table 7). These results demonstrated that taurine treatment during HFD feeding can prevent the disruption of *Per1* expression in pancreatic islets.



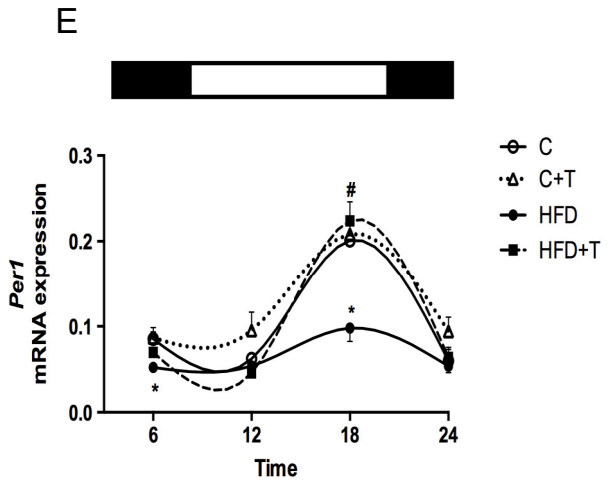


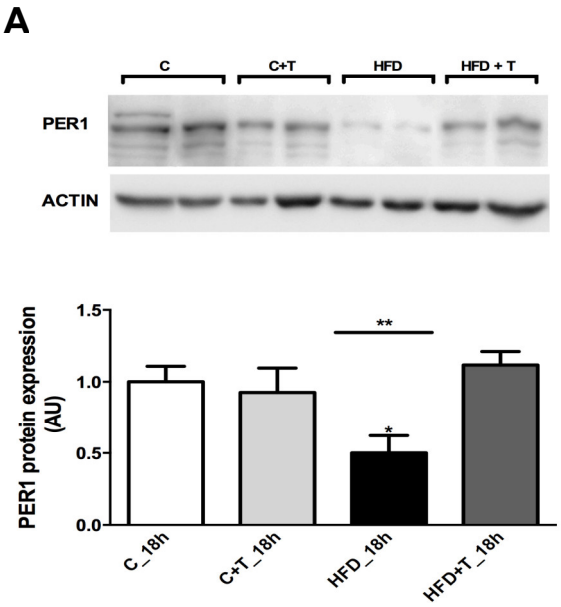
Figure 21. Clock genes expression in islets during 24h.
Pancreatic islets were isolated at different times of day (6:00h, 12:00h, 18:00h and 24:00h) after 10 weeks of taurine treatment. (n=4-5). **(A)** *Clock* gene expression in isolated islets. (C), (C+T), (HFD) and (HFD+T). **(B)** *Rev-erbα* gene expression in islets. **(C)** *Bmal1* gene expression in islets. **(D)** *Per2* gene expression in islets. **(E)** *Per1* gene expression in islets. Data are expressed as mean ± SEM. Differences between C vs C+T ($^{\&\&}$ P<0.01), C vs HFD (* P<0.05) and HFD vs HFD+T ($^{\#}$ P<0.05) (n=6-7 mice per group). The black bars refers on the top of the figures to the dark cycle and the white bars to the light cycle.

4.1.2.10 PER1 protein expression in pancreatic islets

Since taurine modulated the expression of *Per1* in islets, we next quantified protein levels of PER1 in pancreatic islets isolated at 18:00 and 24:00 after 10 weeks of treatment. Confirming our results, the HFD decreased the protein expression of PER1 at 18:00, and taurine restored PER1 protein levels in islets from mice treated with a HFD (Figure 22A). As expected, there was no difference in PER1 protein expression at 24:00, confirming our results on gene expression (P=0.46).

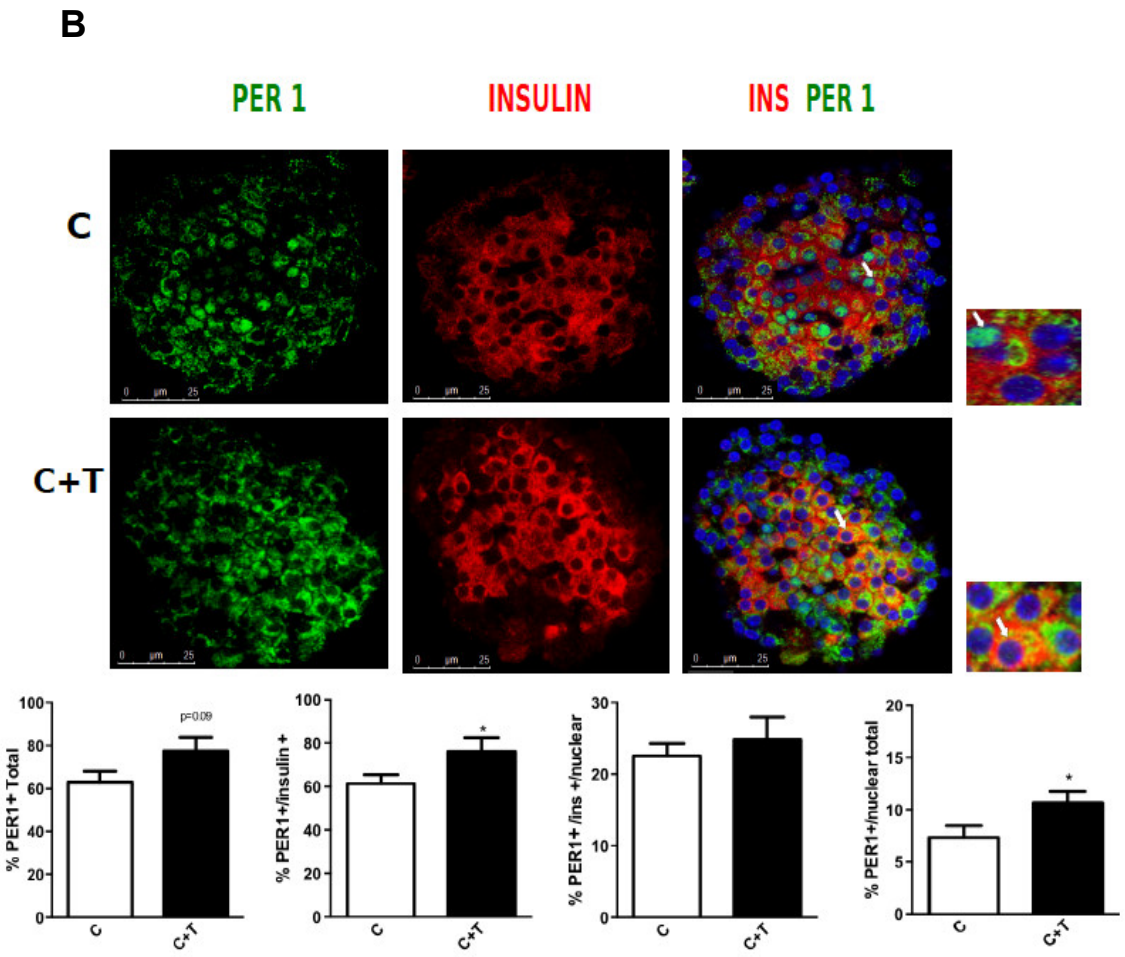
To further validate these results, immunofluorescence was performed in whole fresh isolated pancreatic islets of mice after 10 weeks of treatment. We analysed total

percentage of PER1-positive stained cells within the islet and found a higher tendency for PER1 staining in C+T islets (p=0.09), as compared to control islets (Figure 22B). Moreover, C+T islets showed a statistically significant increase in the percentage of total PER1 in insulin-positive cells when compared with C islets (Figure 22B). Since PER1 is a transcription factor that can be localized in the cytoplasm and/or nucleus, we accessed the cell localization of PER1 in isolated islets. We found similar nuclear PER1 localization in the beta-cells of the C and C+T groups (Figure 22B). However, when measuring nuclear staining in all islet cells, PER1 expression was upregulated by taurine in the control group (Figure 22B).



Percentage of PER1 expression in total number of cells, percentage of PER1 expression in the cytoplasm and the nucleus of insulin-positive cells, percentage of nuclear PER1 in beta-cells and percentage of nuclear PER1 in the total number of cells in chow diet groups. Differences between C (white bars) versus C+T (black bars). Data are expressed as mean ± SEM. Differences between C vs C+T (* P<0.05) (n=15-20 islets analysed per condition per group).

Consistent with our results on gene expression, the HFD downregulated PER 1 protein expression as compared to C islets (P= 0.002). When we compared islets from HFD and HFD+T, we found an increase in total PER1-positive staining with taurine (Figure 22C). Moreover, insulin-positive cells from HFD+T islets presented higher total PER1 labelling than islets from HFD mice (Figure 22C). We observed an increased staining of nuclear PER1 from HFD+T islets and nuclear PER1 staining when analysed in total islet cells, compared to islets from HDF mice (Figure 22C). Thus, these results demonstrated that HFD downregulated PER1 protein and gene expression, whereas taurine upregulated PER1.



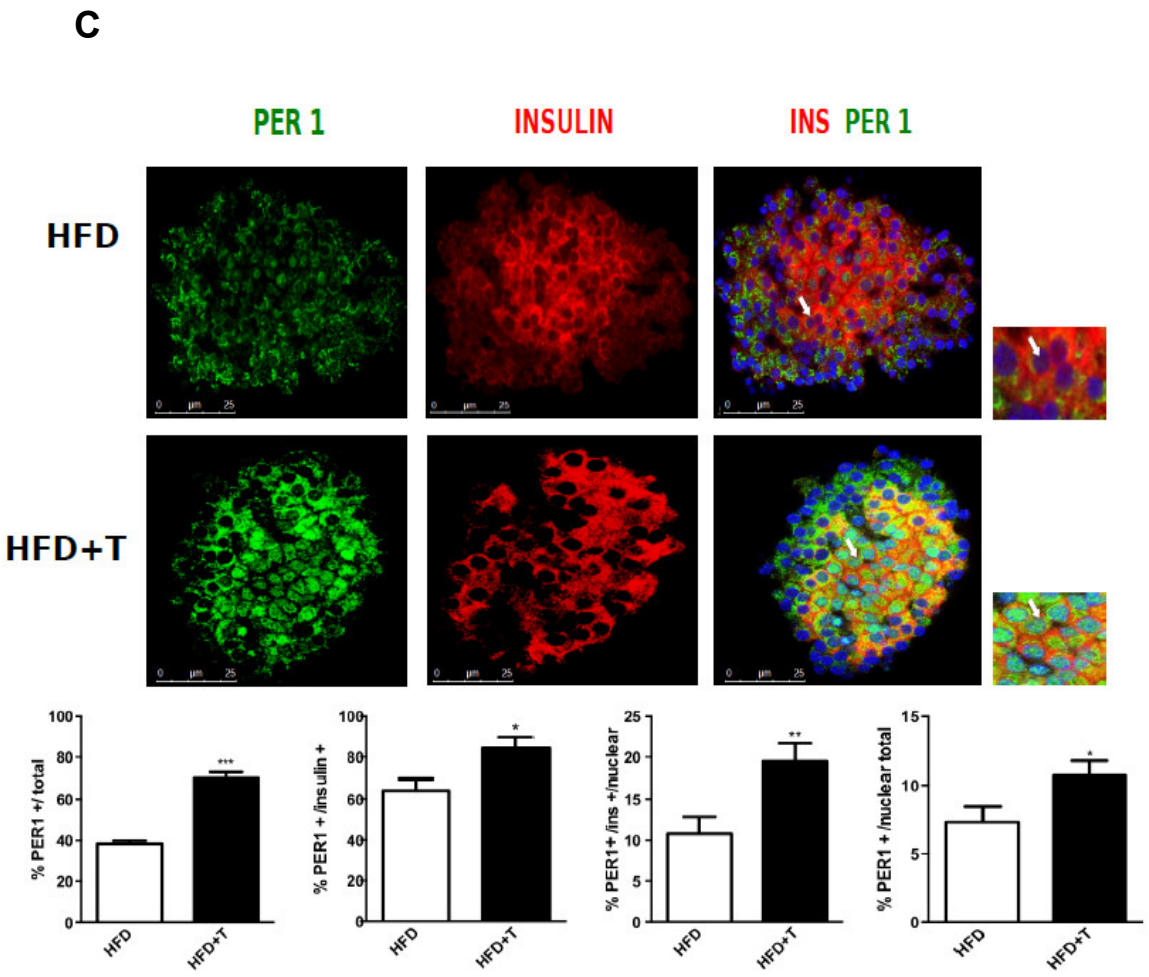


Figure 22. Effects of taurine in the expression of PER1 protein in isolated pancreatic islets.

Pancreatic islets were isolated at 18:00h after 10 weeks of taurine treatment in all experimental groups. Protein expression was detected by western blot and immunofluorescence stained against insulin anti-body (red), PER1 anti-body (green) and nuclear fraction by DAPI (blue). (A) PER1 protein expression normalized by actin. (n=4-5 mice per group). (B) Percentage of PER1 expression in total number of cells, percentage of PER1 expression in the cytoplasm and the nucleus of insulin-positive cells, percentage of nuclear PER1 in beta-cells and percentage of nuclear PER1 in the total number of cells in chow diet groups. Differences between C (white bars) versus C+T (black bars). Data are expressed as mean ± SEM. Differences between C vs C+T (* P<0.05) (n=15-20 islets analysed per condition per group). (C) Percentage of PER1 expression in total number of cells, percentage of PER1 expression in the cytoplasm and the nucleus of insulin-positive cells, percentage of nuclear PER1 in beta-cells and percentage of nuclear PER1 in the total number of cells in high fat diet groups. Differences between HFD (white bars) versus HFD+T (black bars). Data are expressed as mean ± SEM. Differences between HFD vs HFD+T (* P<0.05, **P<0.01, ***P<0.001, n=15-20 islets analysed per condition per group).

Table 6. Cosinor analysis of the 24h expression of metabolic parameters.

	R^2				Mesor							Amplitude							Acrophase						
	C	C+T	HFD	HFD+T	C	C+T	HFD	HFD+T	P Value			C	C+T	HFD	HFD+T	P Value			C	C+T	HFD	HFD+T	P Value		
									C vs C+T	C vs HFD	HFD vs HFD+T					C vs C+T	C vs HFD	HFD vs HFD+T					C vs C+T	C vs HFD	HFD vs HFD+T
Glucose	0,028	0,020	0,00	0,003	123,7	116,0	136,5	121,2	0,01$^{\&\&}$	0,005**	0,001$^{\#\#\#}$	11,6	12,9	12,1	9,4	0,76	0.85	0.25	11,0	23,0000	17,0	17,0	0,001$^{\&\&\&}$	0,96	0,9
Insulin	0,24	0,38	0,01	0,60	1,4	1,5	9,3	2,9	0,87	0,001***	0,007$^{\#\#}$	0,6	0,7	3,3	1,80	0,7	0.001***	0.04$^{\#}$	23,0	23,0	17,0	23,0	0,77	0,35	0,9
Leptin	0,49	0,34	0,06	0,48	3,5	4,6	64,3	38,9	0,11	0,001***	0,007$^{\#\#}$	2,6	2,5	27,4	15,8	0,61	0.001***	0.01$^{\#\#}$	23,0	23,0	17,0	23,0	0,32	0,68	0,2

C vs C+T$^{\&\&}$ p<0,01 $^{\&\&\&}$ p<0,01 C vs HFD ** p<0.05 *** p<0.001 HFD vs HFD+T $^{\#}$ p<0.05 $^{\#\#}$ p<0.01 $^{\#\#\#}$ p<0.001

Cosinor analysis including Goodness of the fit (R2), mesor, amplitude and acrophase of the 24h profiles of plasma glucose, insulin and leptin. Next-Two way anova shows the difference between groups in mesor, amplitude and acrophase respectively. Shown are the means ± SEM.

Table 7. Cosinor analysis of the 24h expression of clock genes.

	R^2				Mesor							Amplitude							Acrophase						
	C	C+T	HFD	HFD+T	C	C+T	HFD	HFD+T	P Value			C	C+T	HFD	HFD+T	P Value			C	C+T	HFD	HFD+T	P Value		
									C vs C+T	C vs HFD	HFD vs HFD+T					C vs C+T	C vs HFD	HFD vs HFD+T					C vs C+T	C vs HFD	HFD vs HFD+T
Rev-erb alpha	0,74	0,82	0,43	0,42	0,17	0,17	0,11	0,08	0,91	0,05*	0,25	0,17	0,18	0,11	0,09	0,67	0,17	0,55	11,0	17,0$^{\&}$	11,0	11,0	0,05$^{\&}$	0,34	0,19
Clock	0,23	0,08	0,46	0,30	0,17	0,24	0,18	0,26	0,13	0,63	0,05$^{\#}$	0,16	0,23	0,16	0,28	0,21	0,89	0,05$^{\#}$	23,0	18,0	23,0	18,0	0,91	0,87	0,8
Bmal1	0,80	0,65	0,39	0,54	0,04	0,47	0,03	0,04	,001$^{\&\&\&}$	0,01**	0,02$^{\#}$	0,04	0,04	0,02	0,03	0,99	0,02*	0,74	6,0	6,0	6,0	23,0	0,57	0,22	0,05$^{\#}$
Per 1	0,46	0,42	0,36	0,60	0,10	0,12	0,06	0,10	0,12	0,05*	0,05$^{\#}$	0,07	0,09	0,03	0,09	0,20	0,04*	0,005$^{\#\#}$	17,0	17,0	18,0	18,0	0,40	0,42	0,50
Per 2	0,41	0,30	0,46	0,36	0,12	0,18	0,12	0,12	0,15	0,85	0,90	0,09	0,13	0,08	0,10	0,31	0,50	0,63	17,0	17,0	19,0	19,0	0,20	0,34	0,34

C vs C+T$^{\&}$ p<0.05 $^{\&\&\&}$ p<0.001 C vs HFD* p<0.05 ** p<0.01 HFD vs HFD+T$^{\#}$ p<0.05 $^{\#\#}$ p<0.01

Cosinor analysis including Goodness of the fit (R^2), mesor, amplitude and acrophase of the 24 h profiles of clock genes in pancreatic islets. Next Two-way anova shows the difference between groups in mesor, amplitude and acrophase respectively. Values shown are the means ± SEM.

4.1.2.11 Expression of genes of lipid metabolism and adipose tissue

We measured the expression of genes involved in lipid metabolism. HFD decreased the expression levels of transcription factors related with fatty acid oxidation *Cpt1α* and acyl-CoA oxidase (*Aco*) compared to control. However, taurine enhanced the levels of these genes. In addition, taurine increased the expression of *Pparγ* which is regulator of *Cpt1α* and *Aco*. In contrast, taurine had no effect on the expression of *C/ebpα* and *C/ebpβ* gene expression that were increased in high fat diet groups. In respect to Hormone sensitive lipase (*Hsl*) which is implicated in triglyceride hydrolysis, HFD displayed downregulation of *Hsl* when compared to controls, on the other hand taurine increased the levels of *Hsl* indicating a role of taurine in adipose lipolysis. On the other hand, *Glut4* is the main glucose transporter isoform in adipose tissue. Glut4 expression was decreased in HFD group whereas taurine restore the levels of Glut4. Taken together, these data suggested that taurine might lead to increased energy fatty acid oxidation, and improvement in glucose homeostasis.

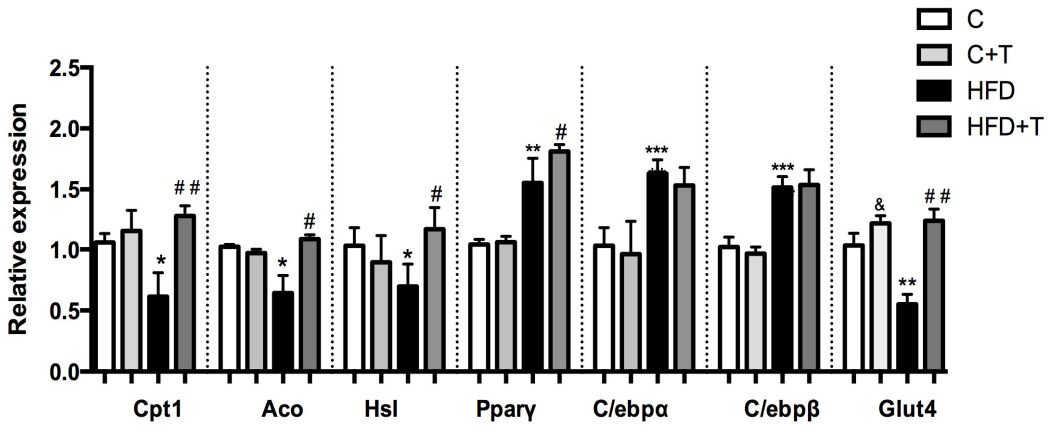


Figure 23. Gene expression of genes implicated in in adipose tissue metabolism after 10 weeks.

Data are expressed as mean ± SEM. Differences between C vs HFD (*p<0.05 **p<0.01, ***p<0.001). C vs C+T ($^{\&}$ p<0.05), HFD vs HFD+T ($^{\#}$ p<0.05, $^{\#\#}$ p<0.01)-

4.1.2.12 Effects of taurine on Nocturnin, a clock controlled gene in adipose tissue.

Nocturnin in adipose tissue has been implicated in lipid metabolism, adipocyte differentiation and adipogenesis [68]. We analysed the expression of Nocturnin during 24h in adipose tissue, increasing from 6h to 24h with the highest peak at 18h and 24h in control mice, with a similar expression pattern with C+T. However HFD mice displayed higher levels throughout the day compared to control. Showing the highest peak at 18h and 24h. Taurine decreased the expression levels of Nocturnin at the end of the light cycle. Indicating that taurine can prevent the disruption of Nocturnin in HFD mice.

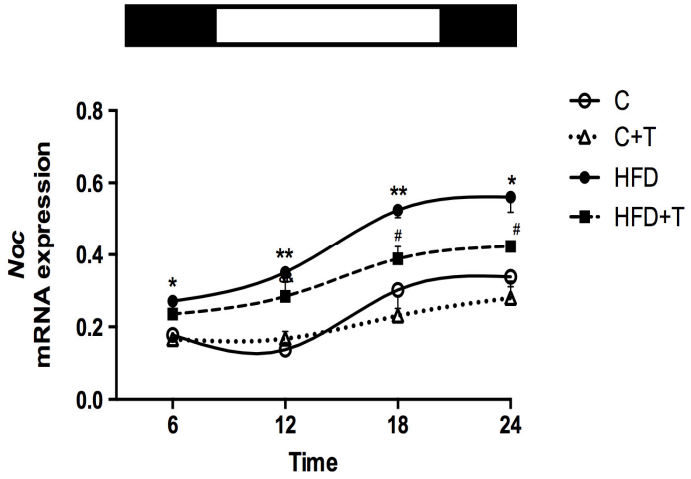


Figure 24. Nocturnin expression in adipose tissue during 24h.
Noc gene expression in adipose tisse. (C), (C+T), (HFD) and (HFD+T). Data are expressed as mean ± SEM. Differences between (*,# P<0.05, **P<0.01)

4.1.2.13 Effects of taurine on clock gene expression on visceral adipose tissue.

We next checked whether taurine could modulate the daily pattern of *Bmal1*, *Clock*, *Rev-erbα*, *Per1* and *Per2* expression in visceral adipose tissue. The expression of *Clock* was similar in control or C+T islets (Figure 25A). However, HFD disrupted *Clock* expression in visceral adipose tissue by increasing *Clock* expression at 6:00 and at 18 and 24:00. Taurine treatment was able to restore the *Clock* expression at 18:00 and 24 in mice fed with HFD (Figure 25A). *Bmal1* expression in control group had a peak of expression at 6:00 and 24:00, and these levels were decreased in C+T group at 6:00 and 24:00. Also *Bmal1* was down regulated by HFD at 12:00 and 24:00 but HFD+T treatment had no effect on *Bmal1* expression at these time points (Figure 25B). The expression of *Rev-erbα* in visceral adipose tissue was decreased at 6:00 and increased at 24:00 by HFD (Figure 25C) and taurine treatment could normalize *Rev-erbα* mRNA levels at 6:00 and 24:00. *Per1* expression was also decreased at 6:00 in visceral adipose tissue from mice fed a HFD but in this case, taurine treatment had no effect on *Per1* expression in visceral adipose tissue (Figure 25D). Finally, C+T increased the expression of *Per2* at 24:00 compared to control. HFD disrupted *Per2* expression by upregulating the expression of this gene at 6:00 and 24:00 whereas taurine treatment restored *Per2* expression at both 6 and 24:00 in mice treated with HFD (Figure 25E).Thus, taurine treatment during HFD feeding can prevent the disruption of *Clock*, *Rev-erbα* and *Per2* expression in visceral adipose tissue.

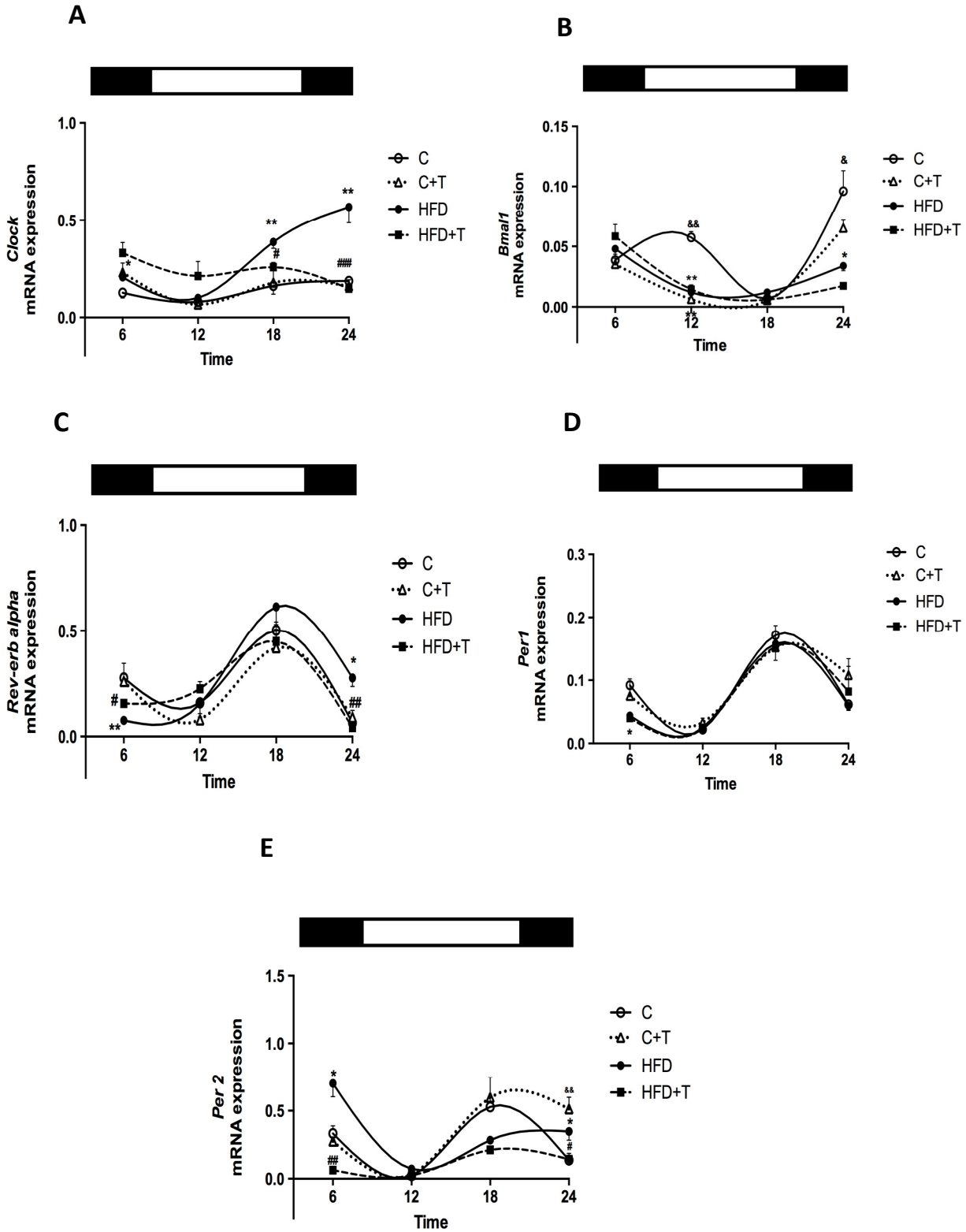


Figure 25. Clock genes expression in adipose tissue during 24h.
Expression of clock genes in visceral adipose tissue at different times of day (6:00h, 12:00h, 18:00h and 24:00h) after 10 weeks of taurine treatment. (n=4-5). **(A)** *Clock* gene expression in adipose tissue (C), (C+T), (HFD) and (HFD+T). **(B)** *Rev-erbα*

gene expression in adipose tissue. **(C)** *Bmal1* gene expression in islets. **(D)** *Per1* gene expression in adipose tissue. **(E)** *Per2* gene expression in adipose tissue. Data are expressed as mean ± SEM. Differences between C vs C+T ($^{\&}$P<0.05, $^{\&\&}$ P<0.01), C vs HFD (* P<0.05, **P<0.01) and HFD vs HFD+T ($^{\#}$ P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001) (n=6-7 mice per group). The black bars refer on the top of the figures to the dark cycle and the white bars to the light cycle.

5. DISCUSSION

Disruptions of biological clocks and their desynchronization are related to altered activity, feeding cycles and hence altered body homeostasis. Experimental and epidemiological studies suggest a strong association between altered circadian behaviour and the development of metabolic diseases. The role of the circadian clock has increased clinical interest based on epidemiological data connecting lifestyles related to circadian disruption and increased risk of T2DM and obesity [239]. Otherwise nutrients have been identified as important factors to influence circadian rhythms at both molecular and behavioral levels. Understand the relationship between functional nutrients, feeding and the circadian clock is of relevant importance for metabolic health, and to aim strategies for obesity prevention and treatment. The impact of taurine on circadian rhythms has not been studied previously. Although taurine data from several studies revealed many important and therapeutically effects of physiological significance, indicating the nutritional importance of taurine to prevent lifestyle-related diseases. In the present work, we used a model of diet-induced obesity, as a high-fat diet affects multiple behavioural and molecular circadian rhythms [126,240]. We showed that taurine prevented multiple effects in diet-induced obesity in mice. Taurine is considered a non-toxic amino acid and is easily supplemented into daily diets. First we examined the effects of short-term of 1-week consumption of a high-fat diet to rule out the taurine's body effects in mice phenotype.

During the 1th week HFD mice were glucose intolerant and showed decreased insulin sensitivity, in accordance with previous studies where glucose intolerance is induced already at 3 days of HFD [241,242]. No increase in body weight gain was observed despite the higher energy consumption with HFD. Results of many studies have shown that within the 1th week changes in body weight increase in HFD mice, depending also on the composition of diet, experimental design, the different mice genetic background, age and strain [241,243].

On the other hand, taurine had no effect on glucose tolerance, insulin sensitivity, body weight and visceral fat at this early stage of HFD, however decreases food intake during day and night already at 1 week preceding the changes in body weight. However we found that HFD increases food intake at the first week of treatment and at 10th week during the light and dark cycles. Previous studies have shown that at 1 week of HFD there is an increased food intake during the normal resting phase in mice [126]. It has been suggested that shifting feeding behavior is an important factor influencing body weight and energy metabolism. Different studies show that eating during the inactive phase (light) leads to increased adiposity, which could be related to decreased energy expenditure, its has been studied that mice fed with a HFD only during light phase over a period of 6 weeks gained 2.5-fold more weight than mice given the same diet during the dark phase, despite caloric intake and physical activity were identical between groups. Although many metabolic parameters are influenced by these changes the contributing mechanisms have yet to be determined [244,245].

We measured the expression of clock genes at 1 week of HFD, although gene expression was measured only at one point, there were no differences in Clock, Bmal1, Rev-erbα, Per1. Nevertheless, further studies should be carried out to analize if the changes in clock gene expression precedes metabolic abnormalities.

In our results taurine treatment prevents HFD–increased food intake during both light and dark period at 1st and at 10th week of treatment, suggesting a central effect to modulate food intake, It has been shown that taurine reduces food intake preventing leptin resistance in hypothalamus and by overexpressing anorexigenic neuropeptides [246], also it was studied that central administration of taurine decreases NPY expression, even administered alone without insulin, the anorexic effect of taurine had a similar magnitude to the effect produced by either insulin [247].

Hyperinsulinemia and insulin resistance are two characteristics of obesity that have been proposed to contribute to its detrimental effects on health. Circulating insulin levels are intimately related to systemic insulin responsiveness [248] . After 10 weeks of HFD and taurine, HFD increased body weight and visceral fat, animals became glucose intolerant and insulin resistant. Despite the high circulating insulin levels, obese mice were only able to maintain plasma glucose levels within the normoglycemic range in the fasted state but not during fed conditions or during an i.p. glucose challenge. Taurine supplementation prevents the increase in body weight, evident as lower visceral fat, ameliorated glucose intolerance and enhanced insulin sensitivity consistent with different studies describing anti-obesity actions of this aminoacid, also in different experimental models of insulin resistance where taurine supplementation normalizes plasma insulin, glucose levels and increases insulin sensitivity [249–252]. Being likely that effects of taurine are detectable in long-term treatments in the context of obesity and diabetes [253–255]. In this sense, several groups have analyzed the mechanisms underlying the improvement of peripheral insulin sensitivity by taurine. It has been showed that taurine had an improvement in glucose homeostasis and insulin action in liver and muscle on the insulin receptor, enhancing insulin action [256]. Moreover, taurine also prevented insulin resistance in liver induced by intravenous infusion of fatty acids, leading to an inhibition of oxidative stress caused by fatty acids which impairs insulin signaling [257]. In addition, the effects of taurine in adipose tissue have been studied. Taurine ameliorated the hyperglycemia in part through inducing the production of antiinflamatory mediators which supresses the development of insulin resistance in adipose tissue [258]. Likewise in adipocytes, taurine mediated the polarization of macrophages preventing insulin resistance independently of decrease in body weight [259]. Thus indicating that taurine has a beneficial effect at least in part ameliorating peripheral sensitivity.

In addition daily variations in glucose, insulin and leptin are subjected to circadian control during normal conditions and are disrupted in diabetes and obesity. Our results confirm the increase insulin and leptin during both light and dark period with HFD as previously shown [126]. The mesor and amplitude values showed that taurine decreased insulin and leptin levels throughout 24-h in mice treated with a HFD. Insulin displays a low-amplitude daily rhythm which is strongly influenced by food ingestion that enhances insulin release [260], nonetheless, in vitro pancreatic islets release insulin in a circadian time scale, thus illustrating the endogenous nature of these oscillations [207].

Disrupted adipokine circadian patterns, in the case of leptin may have an impact on the appetite, food intake, and energy balance. The increase in leptin levels during the night may mean it acts as a satiety hormone, favoring fasting and nocturnal rest. Obesity is correlated not only with high levels of leptin but also with a reduction in the amplitude of the rhythm and attenuation of rhythmicity [261]. Another interesting aspect is that adipocytokines produced by adipose tissue, such as leptin, adiponectin and resistin, not only show clear circadian rhythms in their plasma concentration but their rhythmicity is strongly attenuated or even absent in obese individuals [262]. The mechanisms implicated in the preventive effects of taurine on leptin are not well known, It could be possible that taurine, by regulating insulin levels, could normalize the daily pattern of leptin, In fact it has been shown that leptin levels were shown to be dependent on insulin levels [263,264]. Insulin and leptin signaling constitute the adipoinsular axis, which contributes to the regulation of nutrient and energy balance in the body. Leptin suppresses insulin secretion in a negative feedback loop where insulin stimulates the release of leptin [265,266].

Since taurine acts regulating central hypothalamic signaling, additional studies will be needed to disregard the effect of taurine on leptin signaling for example in adipose tissue. The sympathetic input to adipocytes has been reported to be essential for the

regulation of daily rhythms in leptin release from adipose tissue [55]. Moreover, disruption in fatty acid oxidation, is associated with decreased Cpt1*α* and Aco expression or activity, keys enzymes in fatty-acid β-oxidation, in our results taurine increases the mRNA levels of *Cpt1α* and *Aco* in adipose tissue of HFD mice. It has been shown that in HFD rats fed with taurine displayed enhanced *Cpt1α* activity and decreased hepatic fatty acid esterification [267]. Disruption in fatty acid oxidation, associated with decreased *Cpt1* expression or activity, has been reported in humans and in rodents with non-alcoholic fatty liver disease *(*NAFLD) [268,269]. We further checked the levels of *Pparγ*, a transcription factor, predominantly expressed in adipose tissue that plays a key role in adipocyte differentiation, lipid storage, and glucose homeostasis [270].

In our results taurine treatment increases *Pparγ* levels in adipose tissue in HFD mice, the precise role of taurine on *Pparγ* it is not known, since extensive genes directly and indirectly are modulated by *Pparγ* as well [271]. In addition to its role in adipocyte differentiation and lipid metabolism, *Pparγ* in adipose tissue plays an important role in glucose homeostasis affecting insulin sensitivity in other tissues. In obesity and type 2 diabetes, *Glut4* is downregulated in adipose tissue [272]. In our results, restoring *Glut4* levels in adipose tissue by taurine might improve insulin sensitivity, indeed has been shown that *Pparγ* increased the expression of *Glut4* [273]. Considering that these factors probably act through distinct signalling pathways and different, although overlapping, tissue targets, several different mechanisms may be involved in achieving the insulin-sensitizing effect.

Multiple lines of evidence suggest a close relationship between circadian rhythms and adipose biology. Obesity is associated with circadian rhythms implicating the circadian clock in body weight control. Analysis of adipose tissue has revealed robust 24-h rhythms of clock gene expression in rodents and humans and a functional role in lipid

metabolism [53,274]. We analysed the daily pattern of clock genes in visceral adipose tissue, in our results impaired 24h clock genes expression from obese mice. HFD modified *Clock, Bmal1, Rev-erbα, Per 1* and *Per* 2, taurine change the expression of *Clock, Rev-erbα* and *Per2*. These clock genes are related in adipogenesis [63,65,274], This suggests that HFD leads to obesity and affects the molecular clock function in peripheral tissues. Such changes could be associated with altered diurnal profiles of leptin, glucose, insulin; thus, this is the first evidence showing taurine could modulate clock genes in adipose tissue. Nevertheless future research would be needed to elucidate the effects of taurine on adipose clocks and its chrono-therapeutic approach for obesity.

On the other hand, insulin secretory response shows robust circadian variation in rodents and humans. Although we could not detect a 24h pattern of insulin levels in vivo, we performed glucose-stimulated insulin secretion in isolated islets from 10 weeks with HFD and taurine throughout the 24-h cycle, insulin secretion changed according to the time of day. Insulin secretion in control islets stimulated with glucose was higher during the active phase and reduced during the light cycle, with enhanced circadian insulin secretion in islets from C+T group. In HFD islets, the 24-h pattern of glucose stimulated insulin secretion was altered during the light cycle, consistent with the increase in food intake during the light cycle in these mice. Surprisingly, taurine treatment abolished the daily pattern of insulin secretion in isolated islets from HFD mice. The reason is not known, but it could be possible that taurine could modulate metabolic genes involved in the regulation of insulin secretion and beta cell function.

During obesity, HFD progressively induces in the pancreas a variety of metabolic disorders, including insulin resistance, hyperinsulinemia, and pancreatic islet hypertrophy [275]. It is known that insulin resistance adaptively increases insulin secretion

and compensatory adaptations in the pancreatic beta cells that usually allow for higher pancreatic insulin release in order to maintain normoglycemic values [276].

We showed that isolated islets from obese mice displayed higher insulin gene expression as well as increased secretory output in response to glucose compared with controls. However taurine normalized insulin secretion *in vivo* and *in vitro*. Indeed, it has been demonstrated that taurine prevented insulin hypersecretion in leptin deficient mice [277] and prevents islet hypersecretion and hypertrophy in obese swiss mice [278]. Although the mechanisms for this compensations are not well known, it has been shown that taurine could act through to diverse mechanisms, by the insulin signalling pathway and by increasing the influx of intracellular calcium improved beta-cell responsiveness to glucose in normal and prediabetic rodents [279, 280].

More over, we found that taurine upregulates *Pparγ* in pancreatic islets. Pparγ activation in beta cells has been associated with a variety of survival effects arising in the context of diabetes, pharmacologic Pparγ activation has been shown to protect beta cells against glucose, lipid, cytokine- induced activation of numerous stress pathways [281 282].

The importance of adequate amount of taurine for normal cell development and function is shown in rodents by the numerous pathological consequences that occur when the transport of taurine into the cells is altered, either by the lack of plasma taurine resulting from dietary deficiency or by different pathologies. Taurine metabolism disturbance is closely linked to obesity. It has been reported that a high fat diet causes a decrease in taurine content, which related to the development of obesity [252]. The taurine transporter (TAUT), which transports taurine from the extracellular space into cells to help maintain a high intracellular taurine content, is widely expressed in various tissues [283–285]. We observed decreased *Cdo, Csad* and *TauT* expression in pancreatic islets during HFD mice, whereas supplementation of taurine restored their levels,

indicating that obesity pertubs the levels of taurine in pancreatic islets. In fact it has been shown that alterations in taurine transport altered beta cell mass, suggesting that taurine play a important role for pancreatic islets function [284,286]. Likewise it has been suggested that a taurine deficiency could predispose as well in detrimental effects per se in several tissues, including heart and liver [287–289]. Since taurine plays a role in maintaining cellular function through the regulation of osmotic balance, oxidative stress, and immune and inflammatory responses, could be possible that its depletion accelerates the progression of metabolic diseases, including obesity and diabetes. Further studies are necessary to clarify the mechanism underlying the impact of taurine deficiency. Previous studies demonstrate that pathology develops if the animal is depleted of taurine stores either through a taurine deficient diet or use taurine transport antagonist.

On the other hand, in our results the clock-controlled gene Nocturnin is expressed in pancreatic islets and adipose tissue, in fact its has been shown that nocturnin display pronounced levels in different tissues during a HFD [66,290]. In addition, overexpression of nocturnin enhances adipogenesis and nocturnin as well as deletion of nocturnin in mice leads to resistance to diet-induced obesity. In our results a notable finding is that taurine normalizes the levels of this gene in HFD mice in adipose tissue and pancreatic islets. In adipose tissue it is still not clear how nocturnin functions regarding regulation of lipid metabolism [68]. It has been shown that Nocturnin binds to Pparγ and enhance its transcriptional activity [291]. As well deletion of nocturnin abolished *Pparγ* oscillation in the liver of mice fed on high-fat diet, accompanied by a decrease in expression of many genes related to lipid metabolism. The specific function of nocturnin in pancreatic islets it is not known, since, is a recently circadian deadenylase that participates by altering target mRNA stability through posttranscriptional regulation at different levels, regulating energy homeostasis, including lipid and carbohydrate metabolism, thus

many open questions remain concerning the taurine effects and the regulation of nocturnin in peripheral tissues and studies to determine the mechanism of action of nocturnin in these tissues.

Moreover, the islet clock exhibits oscillations in clock gene expression throughout the day in different species, and alterations in their expression leads to impairments in insulin secretion. Our results show that HFD disrupts the expression of *Bmal1, Rev-erbα* and *Per1* genes in isolated islets. Whereas taurine supplementation could not restored *Bmal1* and *Rev-erbα* expression. An interesting finding of the present study is that taurine could prevent the down regulation of *Per1* levels caused by a HFD in isolated islets. This was evident in measurements of gene and protein expression, mainly in the in insulin-positive cells. The increase in percentage of PER1 expression in beta cells was detected in the nuclear fraction of these cells, suggesting that PER1 could be a target of taurine. Recently it has been found that *Per 1* and *Per 2* are involved in the development of insulin resistance and inflammation [197]. But the specific role of Per 1 in pancreatic islets it is not known.

Considering the different effects of *in vivo* taurine treatment on whole body metabolism, it is difficult that one mechanism could explain the taurine effects found in this study. However from the present study these data demonstrate that taurine improves disturbances in the 24h pattern of plasma insulin and leptin, as well modulates clock genes in adipose tissue and pancreatic islets. These effects of taurine could be tissue-dependent and peripheral clocks may be possible targets of this amino acid during obesity. Since taurine modulated Nocturnin in both peripheral tissues, adipose tissue and islets, could be interesting to further analyse the crosstalk and mechanistic basis of this regulation.

This is the first evidence that shows that taurine could be a strong target to correct or ameliorate the disturbances in circadian rhythms caused by obesity. Taurine has been

demonstrated to have beneficial effects on obesity and lipid profiles in clinical trials. At present, none of the clinical studies performed so far have the sufficient and statistically determined sample size; therefore, more large-scale, long-term clinical studies are needed to elucidate the usefulness and value of taurine in diabetes and obesity [292].

Additionally, circadian alteration becomes more frequent in modern societies, in recent years, the role of the circadian system in the control of glucose metabolism gained clinical interest based on epidemiological data linking lifestyles related to circadian disruption to increased risks of T2DM and obesity. The circadian homeostasis needs not only balanced nutrient components but also regular timed nutrients, Although the importance of taurine as a physiological agent with pharmacological properties is now recognised, the potential advantages of dietary supplementation with taurine have not as yet been fully exploited and this is an area which could prove to be of benefit , thus, adapting the clock to nutrient status could be advantageous, as an understanding of the molecular mechanisms involved could help develop novel chrono-therapeutical approaches for the prevention and treatment of these diseases.

6. CONCLUSIONS

- Taurine treatment can prevent disturbances of circadian rhythm in food intake that was disrupted by HFD.
- Taurine treatment prevents the impairment in glucose tolerance and improves insulin sensitivity, decreased plasma insulin levels in mice treated with HFD.
- Taurine prevented increase in body weight in mice treated with HFD.
- Taurine treatment prevents disturbances in the circadian rhythms of leptin and insulin,
- Taurine treatment modulates the expression clock genes in peripheral tissues.
- Taurine treatment modulates the expression of Nocturnin in peripheral tissues.

7. REFERENCES

1. Paranjpe, D. A. & Sharma, V. Evolution of temporal order in living organisms. *J. Circadian Rhythms* **3,** 7 (2005).

2. Dunlap, J. C. Molecular Bases for Circadian Clocks. *Cell* **96,** 271–290 (1999).

3. Mills, J. N., Minors, D. S. & Waterhouse, J. M. The circadian rhythms of human subjects without timepieces or indication of the alternation of day and night. *J. Physiol.* **240,** 567–94 (1974).

4. Bass, J. & Takahashi, J. S. Circadian integration of metabolism and energetics. *Science* **330,** 1349–1354 (2010).

5. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* **418,** 935–41 (2002).

6. Golombek, D. A. & Rosenstein, R. E. Physiology of circadian entrainment. *Physiol. Rev.* **90,** 1063–102 (2010).

7. Zheng, B. *et al.* Nonredundant Roles of the mPer1 and mPer2 Genes in the Mammalian Circadian Clock. *Cell* **105,** 683–694 (2001).

8. Kume, K. *et al.* mCRY1 and mCRY2 Are Essential Components of the Negative Limb of the Circadian Clock Feedback Loop. *Cell* **98,** 193–205 (1999).

9. Sato, T. K. *et al.* Feedback repression is required for mammalian circadian clock function. *Nat. Genet.* **38,** 312–319 (2006).

10. Brown, S. A., Kowalska, E. & Dallmann, R. (Re)inventing the circadian feedback loop. *Dev. Cell* **22,** 477–87 (2012).

11. Mohawk, J. A. & Takahashi, J. S. Cell autonomy and synchrony of suprachiasmatic nucleus circadian oscillators. *Trends Neurosci.* **34,** 349–58 (2011).

12. Ralph, Foster, M. R., Davis, R. G., Menaker, F. C. & Michael. Transplanted Suprachiasmatic Nucleus Determines Circadian Period. *Sci. Feb* **23,** (1990).

13. Weaver, D. R. The suprachiasmatic nucleus: a 25-year retrospective. *J. Biol.*

Rhythms **13,** 100–12 (1998).

14. Coomans, C. P. *et al.* The suprachiasmatic nucleus controls circadian energy metabolism and hepatic insulin sensitivity. *Diabetes* **62,** 1102–1108 (2013).

15. La Fleur, S. E., Kalsbeek, A., Wortel, J., Fekkes, M. L. & Buijs, R. M. A daily rhythm in glucose tolerance: A role for the suprachiasmatic nucleus. *Diabetes* **50,** 1237–1243 (2001).

16. Groos, G. & Hendriks, J. Circadian rhythms in electrical discharge of rat suprachiasmatic neurones recorded in vitro. *Neurosci. Lett.* **34,** 283–288 (1982).

17. Bos, N. P. A. & Mirmiran, M. Circadian rhythms in spontaneous neuronal discharges of the cultured suprachiasmatic nucleus. *Brain Res.* **511,** 158–162 (1990).

18. Pickard, G. E. & Sollars, P. J. Intrinsically photosensitive retinal ganglion cells. *Rev. Physiol. Biochem. Pharmacol.* **162,** 59–90 (2012).

19. Guilding, C. & Piggins, H. D. Challenging the omnipotence of the suprachiasmatic timekeeper: are circadian oscillators present throughout the mammalian brain? *Eur. J. Neurosci.* **25,** 3195–3216 (2007).

20. Challet, E., Caldelas, I., Graff, C. & Pévet, P. Synchronization of the molecular clockwork by light- and food-related cues in mammals. *Biol. Chem.* **384,** 711–9 (2003).

21. Kiessling, S., Sollars, P. J. & Pickard, G. E. Light stimulates the mouse adrenal through a retinohypothalamic pathway independent of an effect on the clock in the suprachiasmatic nucleus. *PLoS One* **9,** e92959 (2014).

22. Dibner, C., Schibler, U. & Albrecht, U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* **72,** 517–49 (2010).

23. Saeb-Parsy *et al.* Neural Connections of Hypothalamic Neuroendocrine Nuclei in the Rat. *J. Neuroendocrinol.* **12,** 635–648 (2001).

24. Liu, A. C. *et al.* Intercellular coupling confers robustness against mutations in the SCN circadian clock network. *Cell* **129,** 605–16 (2007).

25. Balsalobre, A., Damiola, F. & Schibler, U. A Serum Shock Induces Circadian Gene Expression in Mammalian Tissue Culture Cells. *Cell* **93,** 929–937 (1998).

26. Balsalobre, A. Clock genes in mammalian peripheral tissues. *Cell Tissue Res.* **309,** 193–199 (2002).

27. Richards, J. & Gumz, M. L. Advances in understanding the peripheral circadian clocks. *FASEB J.* **26,** 3602–3613 (2012).

28. Yamazaki, S. *et al.* Resetting central and peripheral circadian oscillators in transgenic rats. *Science* **288,** 682–685 (2000).

29. Tahara, Y. *et al.* In vivo monitoring of peripheral circadian clocks in the mouse. *Curr. Biol.* **22,** 1029–34 (2012).

30. Lamia, K. a, Storch, K.-F. & Weitz, C. J. Physiological significance of a peripheral tissue circadian clock. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 15172–15177 (2008).

31. Gachon, F., Nagoshi, E., Brown, S. A., Ripperger, J. & Schibler, U. The mammalian circadian timing system: from gene expression to physiology. *Chromosoma* **113,** 103–12 (2004).

32. Ando, H. *et al.* Impairment of peripheral circadian clocks precedes metabolic abnormalities in ob/ob mice. *Endocrinology* **152,** 1347–1354 (2011).

33. Eckel-Mahan, K. L. *et al.* Coordination of the transcriptome and metabolome by the circadian clock. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 5541–6 (2012).

34. Gälman, C., Angelin, B. & Rudling, M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology* **129,** 1445–53 (2005).

35. Ma, X., Zhou, Z., Chen, Y., Wu, Y. & Liu, Y. RBP4 functions as a hepatokine

in the regulation of glucose metabolism by the circadian clock in mice. *Diabetologia* **59,** 354–62 (2016).

36. Ma, D. *et al.* The Liver Clock Controls Cholesterol Homeostasis through Trib1 Protein-mediated Regulation of PCSK9/Low Density Lipoprotein Receptor (LDLR) Axis. *J. Biol. Chem.* **290,** 31003–31012 (2015).

37. Rudic, R. D. *et al.* BMAL1 and CLOCK, Two Essential Components of the Circadian Clock, Are Involved in Glucose Homeostasis. *PLoS Biol.* **2,** e377 (2004).

38. Rudic, R. D. *et al.* BMAL1 and CLOCK, Two Essential Components of the Circadian Clock, Are Involved in Glucose Homeostasis. *PLoS Biol.* **2,** e377 (2004).

39. Anne Bugge, Dan Feng, Logan J. Everett, E. A. Rev-Erbα and Rev-Erbβ Protect the Circadian Clock and Metabolic Function. 657–667 (2012). doi:10.1101/gad.186858.112.several

40. Cho, H. *et al.* Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* **485,** 123–127 (2012).

41. Le Martelot, G. *et al.* REV-ERB?? participates in circadian SREBP signaling and bile acid homeostasis. *PLoS Biol.* **7,** 1–12 (2009).

42. Ma, K. *et al.* Circadian dysregulation disrupts bile acid homeostasis. *PLoS One* **4,** (2009).

43. Doi, R., Oishi, K. & Ishida, N. CLOCK regulates circadian rhythms of hepatic glycogen synthesis through transcriptional activation of Gys2. *J. Biol. Chem.* **285,** 22114–21 (2010).

44. Schmutz, I., Ripperger, J. A., Baeriswyl-Aebischer, S. & Albrecht, U. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. *Genes Dev.* **24,** 345–357 (2010).

45. Cinti, S. The adipose organ. *Prostaglandins, Leukot. Essent. Fat. Acids* **73,** 9–15 (2005).

46. Smith, M. M. & Minson, C. T. Obesity and adipokines: effects on sympathetic overactivity. *J. Physiol.* **590,** 1787–801 (2012).

47. Sun, K., Kusminski, C. M. & Scherer, P. E. Adipose tissue remodeling and obesity. *J. Clin. Invest.* **121,** 2094–101 (2011).

48. Suganami, T. & Ogawa, Y. Adipose tissue macrophages: their role in adipose tissue remodeling. *J. Leukoc. Biol.* **88,** 33–39 (2010).

49. Lee, B. C. & Lee, J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1842,** 446–462 (2014).

50. Olefsky, J. M. & Glass, C. K. Macrophages, Inflammation, and Insulin Resistance. *Annu. Rev. Physiol.* **72,** 219–246 (2010).

51. Zvonic, S., Keith, L. & al, et. Characterization of Peripheral Circadian Clocks in Adipose Tissues. *Diabetes* **55,** (2006).

52. Otway, D. T. *et al.* Rhythmic diurnal gene expression in human adipose tissue from individuals who are lean, overweight, and type 2 diabetic. *Diabetes* **60,** 1577–81 (2011).

53. Vieira, E. *et al.* Altered Clock Gene Expression in Obese Visceral Adipose Tissue Is Associated with Metabolic Syndrome. *PLoS One* **9,** e111678 (2014).

54. Nam, D. *et al.* The adipocyte clock controls brown adipogenesis through the TGF-β and BMP signaling pathways. *J. Cell Sci.* **128,** 1835–47 (2015).

55. Kalsbeek, a, Fliers, E., Romijn, J. a & Wortel, J. The Suprachiasmatic Nucleus Generates the Diurnal Changes in Plasma Leptin Levels. **142,** (2001).

56. Kettner, N. M. *et al.* Circadian Dysfunction Induces Leptin Resistance in Mice. *Cell Metab.* **22,** 448–459 (2015).

57. Shimba, S. *et al.* Brain and muscle Arnt-like protein-1 (BMAL1), a component

of the molecular clock, regulates adipogenesis. *Proc. Natl. Acad. Sci.* **102,** 12071–12076 (2005).

58. Shimba, S. *et al.* Deficient of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. *PLoS One* **6,** e25231 (2011).

59. Nam, D. *et al.* Novel Function of Rev-erbα in Promoting Brown Adipogenesis. *Sci. Rep.* **5,** 11239 (2015).

60. Delezie, J. *et al.* The nuclear receptor REV-ERB is required for the daily balance of carbohydrate and lipid metabolism. *FASEB J.* **26,** 3321–3335 (2012).

61. He, W. & He, W. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 15712–7 (2003).

62. Jones, J. & Jones, J. R. Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 6207–12 (2005).

63. Fontaine, C. *et al.* The Orphan Nuclear Receptor Rev-Erb Is a Peroxisome Proliferator-activated Receptor (PPAR) Target Gene and Promotes PPAR -induced Adipocyte Differentiation. *J. Biol. Chem.* **278,** 37672–37680 (2003).

64. Shostak, A., Meyer-Kovac, J. & Oster, H. Circadian regulation of lipid mobilization in white adipose tissues. *Diabetes* **62,** 2195–203 (2013).

65. Grimaldi, B. *et al.* PER2 Controls Lipid Metabolism by Direct Regulation of PPARγ. *Cell Metab.* **12,** 509–520 (2010).

66. Wang, Y. *et al.* Rhythmic expression of Nocturnin mRNA in multiple tissues of the mouse. *BMC Dev. Biol.* **1,** 9 (2001).

67. Gilbert, M. R., Douris, N., Tongjai, S. & Green, C. B. Nocturnin Expression Is Induced by Fasting in the White Adipose Tissue of Restricted Fed Mice. *PLoS One* **6,** e17051 (2011).

68. Kawai, M. *et al.* A circadian-regulated gene, Nocturnin, promotes adipogenesis by stimulating PPAR-gamma nuclear translocation. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 10508–13 (2010).

69. Oster, H. Transcriptional Profiling in the Adrenal Gland Reveals Circadian Regulation of Hormone Biosynthesis Genes and Nucleosome Assembly Genes. *J. Biol. Rhythms* **21,** 350–361 (2006).

70. Balsalobre, A. *et al.* Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **289,** 2344–7 (2000).

71. Pezük, P., Mohawk, J. A., Wang, L. A. & Menaker, M. Glucocorticoids as entraining signals for peripheral circadian oscillators. *Endocrinology* **153,** 4775–4783 (2012).

72. Maury, E., Ramsey, K. M. & Bass, J. Circadian rhythms and metabolic syndrome: From experimental genetics to human disease. *Circ. Res.* **106,** 447–462 (2010).

73. Carroll, T., Raff, H. & Findling, J. W. Late-night salivary cortisol measurement in the diagnosis of Cushing's syndrome. *Nat. Clin. Pract. Endocrinol. Metab.* **4,** 344–350 (2008).

74. Balsalobre, A. *et al.* Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **289,** 2344–7 (2000).

75. Dallmann, R., Touma, C., Palme, R., Albrecht, U. & Steinlechner, S. Impaired daily glucocorticoid rhythm in Per1Brd mice. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **192,** 769–775 (2006).

76. Lamia, K. A. *et al.* Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* **480,** (2011).

77. Nishiyama, K. & Hirai, K. The Melatonin Agonist Ramelteon Induces Duration-Dependent Clock Gene Expression through cAMP Signaling in Pancreatic INS-1 β-Cells. *PLoS One* **9,** e102073 (2014).

78. Houdek, P., Nováková, M., Polidarová, L., Sládek, M. & Sumová, A.

Melatonin is a redundant entraining signal in the rat circadian system. *Hormones and Behavior* **83,** (2016).

79. Mühlbauer, E., Gross, E., Labucay, K., Wolgast, S. & Peschke, E. Loss of melatonin signalling and its impact on circadian rhythms in mouse organs regulating blood glucose. *Eur. J. Pharmacol.* **606,** 61–71 (2009).

80. Chakir, I. *et al.* Pineal melatonin is a circadian time-giver for leptin rhythm in Syrian hamsters. *Front. Neurosci.* **9,** 190 (2015).

81. Gündüz, B. Daily rhythm in serum melatonin and leptin levels in the Syrian hamster (Mesocricetus auratus). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **132,** 393–401 (2002).

82. Yildiz, B. O., Suchard, M. A., Wong, M.-L., McCann, S. M. & Licinio, J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 10434–9 (2004).

83. LeSauter, J., Hoque, N., Weintraub, M., Pfaff, D. W. & Silver, R. Stomach ghrelin-secreting cells as food-entrainable circadian clocks. *Proc. Natl. Acad. Sci.* **106,** 13582–13587 (2009).

84. Kalra, S. P., Bagnasco, M., Otukonyong, E. E., Dube, M. G. & Kalra, P. S. Rhythmic, reciprocal ghrelin and leptin signaling: New insight in the development of obesity. *Regul. Pept.* **111,** 1–11 (2003).

85. Cowley, M. A. *et al.* The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* **37,** 649–661 (2003).

86. Lawrence, C. B., Snape, A. C., Baudoin, F. M. H. & Luckman, S. M. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* **143,** 155–162 (2002).

87. Yannielli, P. C., Molyneux, P. C., Harrington, M. E. & Golombek, D. a. Ghrelin effects on the circadian system of mice. *J. Neurosci.* **27,** 2890–5 (2007).

88. Park, H. K. & Ahima, R. S. Physiology of leptin: energy homeostasis,

neuroendocrine function and metabolism. *Metabolism.* **64,** 24–34 (2015).

89. Bodosi, B. *et al.* Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287,** R1071–R1079 (2004).

90. Xu, B., Kalra, P. S., Farniere, W. G. & Kalra, S. P. Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanoncortin, and adipocyte leptin gene expression and secretion: Effects of food restriction. *Endocrinology* **140,** 2868–2875 (1999).

91. Elimam, A. & Marcus, C. Meal timing, fasting and glucocorticoids interplay in serum leptin concentrations and diurnal profile. *Eur. J. Endocrinol.* **147,** 181–8 (2002).

92. Begg, D. P. & Woods, S. C. The endocrinology of food intake. *Nat. Rev. Endocrinol.* **9,** 584–597 (2013).

93. Grosbellet, E. *et al.* Leptin modulates the daily rhythmicity of blood glucose. *Chronobiol. Int.* **32,** 637–49 (2015).

94. Johnston, J. D., Ordovas, J. M., Scheer, F. A. & Turek, F. W. Circadian Rhythms, Metabolism, and Chrononutrition in Rodents and Humans. *Adv. Nutr. An Int. Rev. J.* **7,** 399–406 (2016).

95. O'Reardon, J. P. *et al.* Circadian eating and sleeping patterns in the night eating syndrome. *Obes. Res.* **12,** 1789–1796 (2004).

96. Sim, L. A. *et al.* Identification and Treatment of Eating Disorders in the Primary Care Setting. *Mayo Clin. Proc.* **85,** 746–751 (2010).

97. Wang, J. B. *et al.* Timing of energy intake during the day is associated with the risk of obesity in adults. *J. Hum. Nutr. Diet.* **27,** 255–262 (2014).

98. Westerterp-Plantenga, M. S., lJedema, M. J. & Wijckmans-Duijsens, N. E. The role of macronutrient selection in determining patterns of food intake in obese and non-obese women. *Eur. J. Clin. Nutr.* **50,** 580–91 (1996).

99. Ma, Y. *et al.* Association between eating patterns and obesity in a free-living US adult population. *Am. J. Epidemiol.* **158,** 85–92 (2003).

100. Colles, S. L., Dixon, J. B. & O'Brien, P. E. Night eating syndrome and nocturnal snacking: association with obesity, binge eating and psychological distress. *Int. J. Obes. (Lond).* **31,** 1722–30 (2007).

101. Hatori, M. *et al.* Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab.* **15,** 848–860 (2012).

102. Garaulet, M. *et al.* Timing of food intake predicts weight loss effectiveness. *Int. J. Obes. (Lond).* **37,** 604–11 (2013).

103. Garaulet, M. *et al.* PERIOD2 Variants Are Associated with Abdominal Obesity, Psycho-Behavioral Factors, and Attrition in the Dietary Treatment of Obesity. *J. Am. Diet. Assoc.* **110,** 917–921 (2010).

104. Schibler, U., Ripperger, J. & Brown, S. A. Peripheral Circadian Oscillators in Mammals: Time and Food. *J. Biol. Rhythms* **18,** 250–260 (2003).

105. Mistlberger, R. E. & Antle, M. C. Entrainment of circadian clocks in mammals by arousal and food. *Essays Biochem.* **49,** 119–36 (2011).

106. Verwey, M. & Amir, S. Food-entrainable circadian oscillators in the brain. *Eur. J. Neurosci.* **30,** 1650–1657 (2009).

107. Merkestein, M. *et al.* Ghrelin Mediates Anticipation to a Palatable Meal in Rats. *Obesity* **20,** 963–971 (2012).

108. Angeles-Castellanos, M., Salgado-Delgado, R., Rodr??guez, K., Buijs, R. M. & Escobar, C. Expectancy for food or expectancy for chocolate reveals timing systems for metabolism and reward. *Neuroscience* **155,** 297–307 (2008).

109. Minokoshi, Y. *et al.* AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428,** 569–74 (2004).

110. Hardie, D. G., Ross, F. a & Hawley, S. a. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* **13,** 251–62 (2012).

111. Lamia, K. A. *et al.* AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* **326,** 437–40 (2009).

112. Burgos, E. S. NAMPT in regulated NAD biosynthesis and its pivotal role in human metabolism. *Curr. Med. Chem.* **18,** 1947–1961 (2011).

113. Yang, Z.-H., Miyahara, H., Takeo, J. & Katayama, M. Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. *Diabetol. Metab. Syndr.* **4,** 1–10 (2012).

114. Glenn, P. F., Chalkiadaki, A. & Guarente, L. Sirtuins mediate mammalian metabolic responses to nutrient availability. *Nat. Publ. Gr.* **8,** (2012).

115. Nakahata, Y. *et al.* The NAD+-Dependent Deacetylase SIRT1 Modulates CLOCK-Mediated Chromatin Remodeling and Circadian Control. *Cell* **134,** 329–340 (2008).

116. Ramsey, K. M. *et al.* Circadian Clock Feedback Cycle Through NAMPT-Mediated NAD+ Biosynthesis. *Science (80-.).* **324,** 651–654 (2009).

117. Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M. & Sassone-Corsi, P. Circadian Control of the NAD+ Salvage Pathway by CLOCK-SIRT1. *Science (80-.).* **324,** 654–657 (2009).

118. Sahar, S., Nin, V., Barbosa, M. T., Chini, E. N. & Sassone-Corsi, P. Altered behavioral and metabolic circadian rhythms in mice with disrupted NAD + oscillation. *Aging (Albany. NY).* **3,** 794–802 (2011).

119. Liu, C., Li, S., Liu, T., Borjigin, J. & Lin, J. D. Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. *Nature* **447,** 477–481 (2007).

120. Cantó, C. *et al. Interdependence of AMPK and SIRT1 for Metabolic*

Adaptation to Fasting and Exercise in Skeletal Muscle. *Cell Metabolism* **11,** (2010).

121. Froy, O., Chapnik, N., Miskin, R. & Long-lived, R. M. Long-lived MUPA transgenic mice exhibit pronounced circadian rhythms. (2006). doi:10.1152/ajpendo.00140.2006.

122. Hara, R. *et al.* Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells* **6,** 269–78 (2001).

123. Mendoza, J. Feeding Cues Alter Clock Gene Oscillations and Photic Responses in the Suprachiasmatic Nuclei of Mice Exposed to a Light/Dark Cycle. *J. Neurosci.* **25,** 1514–1522 (2005).

124. Iwanaga, H. *et al.* Per2 gene expressions in the suprachiasmatic nucleus and liver differentially respond to nutrition factors in rats. *JPEN. J. Parenter. Enteral Nutr.* **29,** 157–61

125. Mendoza, J., Pévet, P. & Challet, E. High-fat feeding alters the clock synchronization to light. *J. Physiol.* **586,** 5901–5910 (2008).

126. Kohsaka, A. *et al.* High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice. *Cell Metab.* **6,** 414–421 (2007).

127. Mendoza, J., Angeles-Castellanos, M. & Escobar, C. A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *Eur. J. Neurosci.* **22,** 2855–2862 (2005).

128. Tahara, Y., Otsuka, M., Fuse, Y., Hirao, A. & Shibata, S. Refeeding after fasting elicits insulin-dependent regulation of Per2 and Rev-erbα with shifts in the liver clock. *J. Biol. Rhythms* **26,** 230–40 (2011).

129. Adamovich, Y. *et al.* Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. *Cell Metab.* **19,** 319–30 (2014).

130. Eckel-Mahan, K. L. *et al.* Reprogramming of the circadian clock by nutritional challenge. *Cell* **155,** 1464–1478 (2013).

131. Kalsbeek, A., La Fleur, S. & Fliers, E. Circadian control of glucose metabolism. *Mol. Metab.* **3,** 372–383 (2014).

132. Lozano, I. *et al.* High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutr. Metab. (Lond).* **13,** 15 (2016).

133. Brown, C. M., Dulloo, A. G., Yepuri, G. & Montani, J.-P. Fructose ingestion acutely elevates blood pressure in healthy young humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294,** 730–737 (2008).

134. Morris, M. *et al.* Timing of fructose intake: An important regulator of adiposity. *Clin. Exp. Pharmacol. Physiol.* **39,** 57–62 (2012).

135. Oike, H., Nagai, K., Fukushima, T., Ishida, N. & Kobori, M. Feeding Cues and Injected Nutrients Induce Acute Expression of Multiple Clock Genes in the Mouse Liver. *PLoS One* **6,** e23709 (2011).

136. Hirao, A., Tahara, Y., Kimura, I. & Shibata, S. A balanced diet is necessary for proper entrainment signals of the mouse liver clock. *PLoS One* **4,** e6909 (2009).

137. Oishi, K., Uchida, D. & Itoh, N. Low-Carbohydrate, High-Protein Diet Affects Rhythmic Expression of Gluconeogenic Regulatory and Circadian Clock Genes in Mouse Peripheral Tissues. *Chronobiol. Int.* **29,** 799–809 (2012).

138. Brager, A. J., Ruby, C. L., Prosser, R. A. & Glass, J. D. Chronic Ethanol Disrupts Circadian Photic Entrainment and Daily Locomotor Activity in the Mouse. *Alcohol. Clin. Exp. Res.* no-no (2010). doi:10.1111/j.1530-0277.2010.01204.x

139. Filiano, A. N. *et al.* Chronic Ethanol Consumption Disrupts the Core Molecular Clock and Diurnal Rhythms of Metabolic Genes in the Liver without Affecting the Suprachiasmatic Nucleus. *PLoS One* **8,** e71684 (2013).

140. Kuroda, H. *et al.* Meal frequency patterns determine the phase of mouse peripheral circadian clocks. *Sci. Rep.* **2,** 711 (2012).

141. Hamaguchi, Y., Tahara, Y., Kuroda, H., Haraguchi, A. & Shibata, S. Entrainment of mouse peripheral circadian clocks to <24 h feeding/fasting cycles under 24 h light/dark conditions. *Sci. Rep.* **5,** 14207 (2015).

142. Ikeda, Y. *et al.* Feeding and adrenal entrainment stimuli are both necessary for normal circadian oscillation of peripheral clocks in mice housed under different photoperiods. *Chronobiol. Int.* **32,** 195–210 (2015).

143. Pivovarova, O. *et al.* Changes of Dietary Fat and Carbohydrate Content Alter Central and Peripheral Clock in Humans. *J. Clin. Endocrinol. Metab.* **100,** 2291–302 (2015).

144. Challet, E. Interactions between light, mealtime and calorie restriction to control daily timing in mammals. *J. Comp. Physiol. B.* **180,** 631–44 (2010).

145. Oike, H., Kobori, M., Suzuki, T. & Ishida, N. *Caffeine lengthens circadian rhythms in mice*. *Biochemical and Biophysical Research Communications* **410,** (2011).

146. Pifferi, F. *et al.* Effects of resveratrol on daily rhythms of locomotor activity and body temperature in young and aged grey mouse lemurs. *Oxid. Med. Cell. Longev.* **2013,** 187301 (2013).

147. Gutierrez-Monreal, M. A., Cuevas-Diaz Duran, R., Moreno-Cuevas, J. E. & Scott, S.-P. A Role for 1 ,25-Dihydroxyvitamin D3 in the Expression of Circadian Genes. *J. Biol. Rhythms* **29,** 384–388 (2014).

148. Ribas-Latre, A. *et al.* Dietary proanthocyanidins modulate melatonin levels in plasma and the expression pattern of clock genes in the hypothalamus of rats. *Mol. Nutr. Food Res.* **59,** 865–878 (2015).

149. Furutani, A. *et al.* Fish oil accelerates diet-induced entrainment of the mouse peripheral clock via GPR120. *PLoS One* **10,** 1–19 (2015).

150. Bouckenooghe, T., Remacle, C. & Reusens, B. Is taurine a functional nutrient? *Curr. Opin. Clin. Nutr. Metab. Care* **9,** 728–733 (2006).

151. Heird, W. C. Taurine in neonatal nutrition - revisited. *Arch. Dis. Child. - Fetal*

Neonatal Ed. **89,** F473–F474 (2004).

152. Huxtable, R. J. Physiological actions of taurine. *Physiol. Rev.* **72,** 101–63 (1992).

153. Yamori, Y. *et al.* Taurine in health and diseases: consistent evidence from experimental and epidemiological studies. *J. Biomed. Sci.* **17 Suppl 1,** S6 (2010).

154. Tappaz, M. L. Taurine Biosynthetic Enzymes and Taurine Transporter: Molecular Identification and Regulations. *Neurochem. Res.* **29,** 83–96 (2004).

155. Niewiadomski, J. *et al.* Effects of a block in cysteine catabolism on energy balance and fat metabolism in mice. *Ann. N. Y. Acad. Sci.* **1363,** 99–115 (2016).

156. Hansen, S. H. The role of taurine in diabetes and the development of diabetic complications. *Diabetes. Metab. Res. Rev.* **17,** 330–346 (2001).

157. Chang, Y. Y. *et al.* Preventive effects of taurine on development of hepatic steatosis induced by a high-fat/cholesterol dietary habit. *J. Agric. Food Chem.* **59,** 450–457 (2011).

158. Han, X., Patters, A. B., Jones, D. P., Zelikovic, I. & Chesney, R. W. The taurine transporter: mechanisms of regulation. *Acta Physiol.* **187,** 61–73 (2006).

159. Warskulat, U. *et al.* Taurine deficiency and apoptosis: Findings from the taurine transporter knockout mouse. *Arch. Biochem. Biophys.* **462,** 202–209 (2007).

160. Bouckenooghe, T., Remacle, C. & Reusens, B. Is taurine a functional nutrient? *Curr. Opin. Clin. Nutr. Metab. Care* **9,** 728–733 (2006).

161. Stapleton, P. P., Charles, R. P., Redmond, H. P. & Bouchier-Hayes, D. J. Taurine and human nutrition. *Clin. Nutr.* **16,** 103–108 (1997).

162. Laidlaw, S., Grosvenor, M. & Kopple, J. The taurine content of common

foodstuffs. *J. Parenter. Enter. Nutr.* **14,** 183–188 (1990).

163. Sahar, S., Nin, V., Barbosa, M. T., Chini, E. N. & Sassone-Corsi, P. Altered behavioral and metabolic circadian rhythms in mice with disrupted NAD + oscillation. *Aging (Albany. NY).* **3,** 794–802 (2011).

164. Klein, D. C., Wheler, G. H. & Weller, J. L. Taurine in the pineal gland. *Prog. Clin. Biol. Res.* **125,** 169–81 (1983).

165. Jia, F. *et al.* Taurine Is a Potent Activator of Extrasynaptic GABAA Receptors in the Thalamus. *J. Neurosci.* **28,** 106–115 (2008).

166. Davies, S. K. *et al.* Effect of sleep deprivation on the human metabolome. *Proc. Natl. Acad. Sci.* **111,** 10761–10766 (2014).

167. Rosa, F. T., Freitas, E. C., Deminice, R., Jordão, A. A. & Marchini, J. S. Oxidative stress and inflammation in obesity after taurine supplementation: a double-blind, placebo-controlled study. *Eur. J. Nutr.* **53,** 823–830 (2014).

168. Askwith, T., Zeng, W., Eggo, M. C. & Stevens, M. J. Oxidative stress and dysregulation of the taurine transporter in high-glucose-exposed human Schwann cells: implications for pathogenesis of diabetic neuropathy. *Am. J. Physiol. Endocrinol. Metab.* **297,** E620-8 (2009).

169. Arany, E. *et al.* Taurine supplement in early life altered islet morphology, decreased insulitis and delayed the onset of diabetes in non-obese diabetic mice. *Diabetologia* **47,** 1831–1837 (2004).

170. Das, J. & Sil, P. C. Taurine ameliorates alloxan-induced diabetic renal injury, oxidative stress-related signaling pathways and apoptosis in rats. *Amino Acids* **43,** 1509–1523 (2012).

171. Camargo, R. L. Effects of Taurine Supplementation Upon Food Intake and Central Insulin Signaling in Malnourished Mice Fed on a High-Fat Diet. **776,** 3–12 (2013).

172. Piña-Zentella, G., De La Rosa-Cuevas, G., Vázquez-Meza, H., Piña, E. & De Piña, M. Z. Taurine in adipocytes prevents insulin-mediated H 2o 2

generation and activates Pka and lipolysis. *Amino Acids* **42,** 1927–1935 (2012).

173. Ng, M. *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384,** 766–781 (2014).

174. Chaput, J.-P. *et al.* Risk Factors for Adult Overweight and Obesity: The Importance of Looking Beyond the 'Big Two'. *Obes. Facts* **3,** 2–2 (2010).

175. Ning, G. Decade in review—type 2 diabetes mellitus: At the centre of things. *Nat. Rev. Endocrinol.* **11,** 636–638 (2015).

176. Whiting, D. R., Guariguata, L., Weil, C. & Shaw, J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res. Clin. Pract.* **94,** 311–21 (2011).

177. Eckel, R. H. *et al.* Obesity and type 2 diabetes: What Can be unified and what needs to be individualized? *Diabetes Care* **34,** 1424–1430 (2011).

178. Honma, K. *et al.* Loss of circadian rhythm of circulating insulin concentration induced by high-fat diet intake is associated with disrupted rhythmic expression of circadian clock genes in the liver. *Metabolism* **65,** 482–491 (2016).

179. Prasai, M. J. *et al.* Diurnal variation in vascular and metabolic function in diet-induced obesity: divergence of insulin resistance and loss of clock rhythm. *Diabetes* **62,** 1981–9 (2013).

180. Borengasser, S. J. *et al.* High Fat Diet and In Utero Exposure to Maternal Obesity Disrupts Circadian Rhythm and Leads to Metabolic Programming of Liver in Rat Offspring. **9,** (2014).

181. Pivovarova, O. *et al.* Regulation of the clock genes expression in human adipose tissue by the weight loss. *Int. J. Obes. (Lond).* 899–906 (2016). doi:10.1038/ijo.2016.34

182. Gorman, M. R. Differential effects of multiple short day lengths on body

weights of gonadectomized siberian hamsters. *Physiol. Biochem. Zool.* **76,** 398–405

183. Karlsson, B. H., Knutsson, A. K., Lindahl, B. O. & Alfredsson, L. S. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. *Int. Arch. Occup. Environ. Health* **76,** 424–430 (2003).

184. Buxton, O. M. *et al.* Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci. Transl. Med.* **4,** 129ra43 (2012).

185. Morris, C. J. *et al.* Endogenous circadian system and circadian misalignment impact glucose tolerance via separate mechanisms in humans. *Proc. Natl. Acad. Sci. U. S. A.* **112,** E2225-34 (2015).

186. Scheer, F. A. J. L., Hilton, M. F., Mantzoros, C. S. & Shea, S. A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci.* **106,** 4453–4458 (2009).

187. VanHelder, T., Symons, J. D. & Radomski, M. W. Effects of sleep deprivation and exercise on glucose tolerance. *Aviat. Space. Environ. Med.* **64,** 487–92 (1993).

188. Markwald, R. R. *et al.* Impact of insufficient sleep on total daily energy expenditure, food intake and weight gain. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 5695–5700 (2013).

189. Vgontzas, A. N., Bixler, E. O., Chrousos, G. P. & Pejovic, S. Obesity and sleep disturbances: Meaningful sub-typing of obesity. *Arch. Physiol. Biochem.* **114,** 224–236 (2008).

190. Turek, F. W. *et al.* Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* **308,** 1043–5 (2005).

191. Marcheva, B. *et al.* Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* **466,** 627–31 (2010).

192. Liu, J. *et al.* CLOCK and BMAL1 Regulate Muscle Insulin Sensitivity via

SIRT1 in Male Mice. *Endocrinology* **157,** 2259–69 (2016).

193. Cho, H. *et al.* Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* **485,** 123–7 (2012).

194. Solt, L. A. *et al.* Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. *Nature* **485,** 62–8 (2012).

195. Barclay, J. L. *et al.* High-fat diet-induced hyperinsulinemia and tissue-specific insulin resistance in Cry-deficient mice. *Am. J. Physiol. - Endocrinol. Metab.* **304,** E1053–E1063 (2013).

196. Narasimamurthy, R. *et al.* Circadian clock protein cryptochrome regulates the expression of proin fl ammatory cytokines. *Proc Natl Acad Sci U S A* **109,** 12662–12667 (2012).

197. Xu, H. *et al.* Myeloid cell-specific disruption of Period1 and period2 exacerbates diet-induced inflammation and insulin resistance. *J. Biol. Chem.* **289,** 16374–16388 (2014).

198. Zhao, Y. *et al.* Loss of mPer2 increases plasma insulin levels by enhanced glucose-stimulated insulin secretion and impaired insulin clearance in mice. *FEBS Lett.* **586,** 1306–1311 (2012).

199. Yang, S. *et al.* The role of mPer2 clock gene in glucocorticoid and feeding rhythms. *Endocrinology* **150,** 2153–2160 (2009).

200. Garaulet, M. *et al.* CLOCK gene is implicated in weight reduction in obese patients participating in a dietary programme based on the Mediterranean diet. *Int. J. Obes. (Lond).* **34,** 516–523 (2010).

201. Garaulet, M. *et al.* CLOCK genetic variation and metabolic syndrome risk: modulation by monounsaturated fatty acids. *Am. J. Clin. Nutr.* **90,** 1466–1475 (2009).

202. Garaulet, M. *et al.* PERIOD2 variants are associated with abdominal obesity, psycho-behavioral factors, and attrition in the dietary treatment of obesity. *J. Am. Diet. Assoc.* **110,** 917–21 (2010).

203. Ruano, E. G., Canivell, S. & Vieira, E. REV-ERB ALPHA polymorphism is associated with obesity in the Spanish obese male population. *PLoS One* **9,** e104065 (2014).

204. Goumidi, L. *et al.* Impact of REV-ERB alpha gene polymorphisms on obesity phenotypes in adult and adolescent samples. *Int. J. Obes. (Lond).* **37,** 666–72 (2013).

205. Bolli, G. B. *et al.* Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes* **33,** 1150–3 (1984).

206. La Fleur, S. E., Kalsbeek, a., Wortel, J. & Buijs, R. M. A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *J. Neuroendocrinol.* **11,** 643–652 (1999).

207. Peschke, E. & Peschke, D. Evidence for a circadian rhythm of insulin release from perifused rat pancreatic islets. *Diabetologia* **41,** 1085–1092 (1998).

208. Boden, G., Chen, X. & Urbain, J. L. Evidence for a Circadian Rhythm of Insulin Sensitivity in Patients With NIDDM Caused by Cyclic Changes in Hepatic Glucose Production. (1996).

209. Boden, G., Chen, X. & Polansky, M. Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. *Diabetes* **48,** 2182–8 (1999).

210. Chandra, R. & Liddle, R. A. Neural and hormonal regulation of pancreatic secretion. *Curr. Opin. Gastroenterol.* **25,** 441–446 (2009).

211. Frayn, K. N. *Metabolic Regulation*. (210AD).

212. Ishihara, H., Maechler, P., Gjinovci, A., Herrera, P.-L. & Wollheim, C. B. Islet β-cell secretion determines glucagon release from neighbouring α-cells. *Nat. Cell Biol.* **5,** 330–335 (2003).

213. Bansal, P. & Wang, Q. Insulin as a physiological modulator of glucagon secretion. *Am. J. Physiol. Endocrinol. Metab.* **295,** E751–E761 (2008).

214. Dunning, B. E., Foley, J. E. & Ahrén, B. Alpha cell function in health and disease: Influence of glucagon-like peptide-1. *Diabetologia* **48,** 1700–1713 (2005).

215. Mertz, R. J., Iii, J. F. W., Spencer, B., Johnson, J. H. & Dukes, I. D. Activation of Stimulus-Secretion Coupling in Pancreatic -Cells by. **271,** 4838–4845 (1996).

216. Henquin, J. C. The dual control of insulin secretion by glucose involves triggering and amplifying pathways in ??-cells. *Diabetes Res. Clin. Pract.* **93,** S27–S31 (2011).

217. Henquin, J.-C., Ishiyama, N., Nenquin, M., Ravier, M. A. & Jonas, J.-C. Signals and pools underlying biphasic insulin secretion. *Diabetes* **51 Suppl 1,** S60-7 (2002).

218. Seino, S. *et al.* Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *J. Clin. Invest.* **121,** 2118–25 (2011).

219. MacDonald, P. E. *et al.* A KATP Channel-Dependent Pathway within α Cells Regulates Glucagon Release from Both Rodent and Human Islets of Langerhans. *PLoS Biol.* **5,** e143 (2007).

220. Gromada, J., Franklin, I. & Wollheim, C. B. A-Cells of the Endocrine Pancreas: 35 Years of Research But the Enigma Remains. *Endocr. Rev.* **28,** 84–116 (2007).

221. Menge, B. A. *et al.* Loss of inverse relationship between pulsatile insulin and glucagon secretion in patients with type 2 diabetes. *Diabetes* **60,** 2160–2168 (2011).

222. Mühlbauer, E., Wolgast, S., Finckh, U., Peschke, D. & Peschke, E. Indication of circadian oscillations in the rat pancreas. *FEBS Lett.* **564,** 91–96 (2004).

223. Stamenkovic, J. A. *et al.* Regulation of core clock genes in human islets. *Metabolism.* **61,** 978–85 (2012).

224. Sadacca, L. A., Lamia, K. A., DeLemos, A. S., Blum, B. & Weitz, C. J. An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. *Diabetologia* **54,** 120–124 (2011).

225. Lee, J. *et al.* Bmal1 and β-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced β-cell failure in mice. *Mol. Cell. Biol.* **33,** 2327–38 (2013).

226. Lee, J. *et al.* Bmal1 and β-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced β-cell failure in mice. *Mol. Cell. Biol.* **33,** 2327–38 (2013).

227. Vieira, E. *et al.* The clock gene Rev-erbα regulates pancreatic β-cell function: Modulation by leptin and high-fat diet. *Endocrinology* **153,** 592–601 (2012).

228. Qian, J., Block, G. D., Colwell, C. S. & Matveyenko, A. V. Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. *Diabetes* **62,** 3469–3478 (2013).

229. Ruiter, M. *et al.* The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. *Diabetes* **52,** 1709–15 (2003).

230. Bähr, I., Mühlbauer, E., Schucht, H. & Peschke, E. Melatonin stimulates glucagon secretion in vitro and in vivo. *J. Pineal Res.* **50,** 336–344 (2011).

231. Valladares, M., Obregón, A. M. & Chaput, J.-P. Association between genetic variants of the clock gene and obesity and sleep duration. *J. Physiol. Biochem.* **71,** 855–860 (2015).

232. Garcia-Rios, A. *et al.* Beneficial effect of CLOCK gene polymorphism rs1801260 in combination with low-fat diet on insulin metabolism in the patients with metabolic syndrome. *Chronobiol. Int.* **31,** 401–8 (2014).

233. Kim, H. Y. *et al.* Taurine in drinking water recovers learning and memory in the adult APP/PS1 mouse model of Alzheimer's disease. *Sci. Rep.* **4,** 7467 (2014).

234. Murakami, S., Kondo, Y. & Nagate, T. Effects of long-term treatment with taurine in mice fed a high-fat diet: improvement in cholesterol metabolism and vascular lipid accumulation by taurine. *Adv. Exp. Med. Biol.* **483,** 177–86 (2000).

235. Santos-Silva, J. C. *et al.* Taurine supplementation ameliorates glucose homeostasis, prevents insulin and glucagon hypersecretion, and controls β, α, and δ-cell masses in genetic obese mice. *Amino Acids* (2015). doi:10.1007/s00726-015-1988-z

236. Marcinkiewicz, J. & Schaff, S. W. *Taurine 9*.

237. Pasantes-Morales, H., Quesada, O. & Morán, J. in 209–217 (Springer US, 1998). doi:10.1007/978-1-4899-0117-0_27

238. Stubblefield, J. J., Terrien, J. & Green, C. B. Nocturnin: at the crossroads of clocks and metabolism. *Trends Endocrinol. Metab.* **23,** 326–333 (2012).

239. Laermans, J. & Depoortere, I. Chronobesity: Role of the circadian system in the obesity epidemic. *Obes. Rev.* (2016). doi:10.1111/obr.12351

240. Cano, P. *et al.* Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. *Endocrine* **33,** 118–125 (2008).

241. Williams, L. M. *et al.* The Development of Diet-Induced Obesity and Glucose Intolerance in C57Bl/6 Mice on a High-Fat Diet Consists of Distinct Phases. *PLoS One* **9,** e106159 (2014).

242. Lee, Y. S. *et al.* Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. *Diabetes* **60,** 2474–2483 (2011).

243. Yang, Y. *et al.* Variations in body weight, food intake and body composition after long-term high-fat diet feeding in C57BL/6J mice. *Obesity (Silver Spring).* **22,** 2147–55 (2014).

244. Arble, D. M., Bass, J., Laposky, A. D., Vitaterna, M. H. & Turek, F. W.

Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring).* **17,** 2100–2102 (2009).

245. Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R. M. & Escobar, C. Food Intake during the Normal Activity Phase Prevents Obesity and Circadian Desynchrony in a Rat Model of Night Work. *Endocrinology* **151,** 1019–1029 (2010).

246. Camargo, R. L. *et al.* Taurine supplementation preserves hypothalamic leptin action in normal and protein-restricted mice fed on a high-fat diet. *Amino Acids* **47,** 2419–35 (2015).

247. Solon, C. S. *et al.* Taurine enhances the anorexigenic effects of insulin in the hypothalamus of rats. *Amino Acids* **42,** 2403–2410 (2012).

248. Templeman, N. M., Skovsø, S., Page, M. M., Lim, G. E. & Johnson, J. D. A causal role for hyperinsulinemia in obesity. *J. Endocrinol.* JOE-16-0449 (2017). doi:10.1530/JOE-16-0449

249. Batista, T. M. *et al.* in 129–139 (2013). doi:10.1007/978-1-4614-6093-0_14

250. Branco, R. C. S. *et al.* Long-term taurine supplementation leads to enhanced hepatic steatosis, renal dysfunction and hyperglycemia in mice fed on a high-fat diet. *Adv. Exp. Med. Biol.* **803,** 339–51 (2015).

251. Nardelli, T. R. *et al.* Taurine prevents fat deposition and ameliorates plasma lipid profile in monosodium glutamate-obese rats. *Amino Acids* **41,** 901–908 (2011).

252. Tsuboyama-Kasaoka, N. *et al.* Taurine (2-Aminoethanesulfonic Acid) deficiency creates a vicious circle promoting obesity. *Endocrinology* **147,** 3276–3284 (2006).

253. Kim, K. S. *et al.* Taurine ameliorates hyperglycemia and dyslipidemia by reducing insulin resistance and leptin level in Otsuka Long-Evans Tokushima fatty (OLETF) rats with long-term diabetes. *Exp. Mol. Med.* **44,** 665–73 (2012).

254. Di Leo, M. A. S. *et al.* Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats. *Amino Acids* **27,** 187–191 (2004).

255. Harada, N. *et al.* Taurine alters respiratory gas exchange and nutrient metabolism in type 2 diabetic rats. *Obes Res* **12,** 1077–1084 (2004).

256. Xiao, C., Giacca, A. & Lewis, G. F. Oral taurine but not N-acetylcysteine ameliorates NEFA-induced impairment in insulin sensitivity and beta cell function in obese and overweight, non-diabetic men. *Diabetologia* **51,** 139–46 (2008).

257. El-Batch, M., Hassan, A. M. & Mahmoud, H. a. Taurine is more effective than melatonin on cytochrome P450 2E1 and some oxidative stress markers in streptozotocin-induced diabetic rats. *J. Agric. Food Chem.* **59,** 4995–5000 (2011).

258. Wu, N. *et al.* Taurine prevents free fatty acid-induced hepatic insulin resistance in association with inhibiting JNK1 activation and improving insulin signaling in vivo. *Diabetes Res. Clin. Pract.* **90,** 288–296 (2010).

259. Lin, S. *et al.* Taurine improves obesity-induced inflammatory responses and modulates the unbalanced phenotype of adipose tissue macrophages. *Mol. Nutr. Food Res.* **57,** 2155–2165 (2013).

260. Patton, D. F. & Mistlberger, R. E. Circadian adaptations to meal timing: neuroendocrine mechanisms. *Front. Neurosci.* **7,** 185 (2013).

261. Ando, H. *et al.* Rhythmic messenger ribonucleic acid expression of clock genes and adipocytokines in mouse visceral adipose tissue. *Endocrinology* **146,** 5631–5636 (2005).

262. Lecoultre, V., Ravussin, E. & Redman, L. M. The fall in leptin concentration is a major determinant of the metabolic adaptation induced by caloric restriction independently of the changes in leptin circadian rhythms. *J. Clin. Endocrinol. Metab.* **96,** 1512–1516 (2011).

263. Ahrén. Diurnal variation in circulating leptin is dependent on gender, food

intake and circulating insulin in mice. *Acta Physiol. Scand.* **169,** 325–331 (2000).

264. Patel, B. K., Koenig, J. I., Kaplan, L. M. & Hooi, S. C. Increase in plasma leptin and Lep mRNA concentrations by food intake is dependent on insulin. *Metabolism.* **47,** 603–607 (1998).

265. Barr, V. A., Malide, D., Zarnowski, M. J., Taylor, S. I. & Cushman, S. W. Insulin Stimulates Both Leptin Secretion and Production by Rat White Adipose Tissue. **138,** 4463–4472 (2015).

266. Kieffer, T. J. & Habener, J. F. invited review. (2000).

267. Chen, X. *et al.* Taurine supplementation prevents ethanol-induced decrease in serum adiponectin and reduces hepatic steatosis in rats. *Hepatology* **49,** 1554–1562 (2009).

268. Kohjima, M. *et al.* Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **20,** 351–8 (2007).

269. NOH, H. *et al.* Inhibitory Effect of a *Cirsium setidens* Extract on Hepatic Fat Accumulation in Mice Fed a High-Fat Diet *via* the Induction of Fatty Acid β-Oxidation. *Biosci. Biotechnol. Biochem.* **77,** 1424–1429 (2013).

270. Tontonoz, P. & Spiegelman, B. M. Fat and Beyond: The Diverse Biology of PPARγ. *Annu. Rev. Biochem.* **77,** 289–312 (2008).

271. Waki, H., Yamauchi, T. & Kadowaki, T. [Recent advances in PPARgamma research]. *Nihon Rinsho.* **68,** 181–8 (2010).

272. Atkinson, B. J., Griesel, B. A., King, C. D., Josey, M. A. & Olson, A. L. Moderate GLUT4 overexpression improves insulin sensitivity and fasting triglyceridemia in high-fat diet-fed transgenic mice. *Diabetes* **62,** 2249–58 (2013).

273. Leonardini, A., Laviola, L., Perrini, S., Natalicchio, A. & Giorgino, F. Cross-Talk between PPARgamma and Insulin Signaling and Modulation of Insulin

Sensitivity. *PPAR Res.* **2009,** 818945 (2009).

274. Zhu, Z. *et al.* CLOCK promotes 3T3-L1 cell proliferation via Wnt signaling. *IUBMB Life* **68,** 557–568 (2016).

275. Fridlyand, L. E. & Philipson, L. H. Islet adaptation to insulin resistance: mechanisms and implications for intervention. *Diabetes. Obes. Metab.* **8,** 136–145 (2006).

276. Seino, S., Shibasaki, T. & Minami, K. Review series Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *J Clin. Investig.* **121,** 2118–2125 (2011).

277. Santos-Silva, J. C. *et al.* Taurine supplementation regulates pancreatic islet function in response to potentiating agents in leptin-deficient obese mice. *Adv. Exp. Med. Biol.* **803,** 371–85 (2015).

278. Ribeiro, R. A. *et al.* Taurine supplementation prevents morpho-physiological alterations in high-fat diet mice pancreatic β-cells. *Amino Acids* **43,** 1791–1801 (2012).

279. Vettorazzi, J. F. *et al.* Taurine supplementation increases KATP channel protein content, improving Ca2+ handling and insulin secretion in islets from malnourished mice fed on a high-fat diet. *Amino Acids* **46,** 2123–2136 (2014).

280. Ribeiro, R. a *et al.* Taurine supplementation: involvement of cholinergic/phospholipase C and protein kinase A pathways in potentiation of insulin secretion and Ca2+ handling in mouse pancreatic islets. *Br. J. Nutr.* **104,** 1148–1155 (2010).

281. Rosen, E. D. *et al.* Targeted Elimination of Peroxisome Proliferator-Activated Receptor in Cells Leads to Abnormalities in Islet Mass without Compromising Glucose Homeostasis. **23,** 7222–7229 (2003).

282. Sciences, M. Troglitazone prevents mitochondrial alterations , cell destruction , and diabetes in obese prediabetic rats. **96,** 11513–11518 (1999).

283. Idrissi, A. El *et al.* in 199–206 (2009). doi:10.1007/978-0-387-75681-3_20

284. El Idrissi, A., Boukarrou, L. & L'Amoreaux, W. Taurine supplementation and pancreatic remodeling. *Adv. Exp. Med. Biol.* **643,** 353–8 (2009).

285. Ito, T. *et al.* Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. *J. Mol. Cell. Cardiol.* **44,** 927–937 (2008).

286. Han, X. *et al.* Knockout of the TauT Gene Predisposes C57BL/6 Mice to Streptozotocin-Induced Diabetic Nephropathy. *PLoS One* **10,** e0117718 (2015).

287. Ito, T., Yoshikawa, N., Ito, H. & Schaffer, S. W. Impact of taurine depletion on glucose control and insulin secretion in mice. *J. Pharmacol. Sci.* **129,** 59–64 (2015).

288. Ito, T. *et al.* Tissue Taurine Depletion Alters Metabolic Response to Exercise and Reduces Running Capacity in Mice. *J. Amino Acids* **2014,** 1–10 (2014).

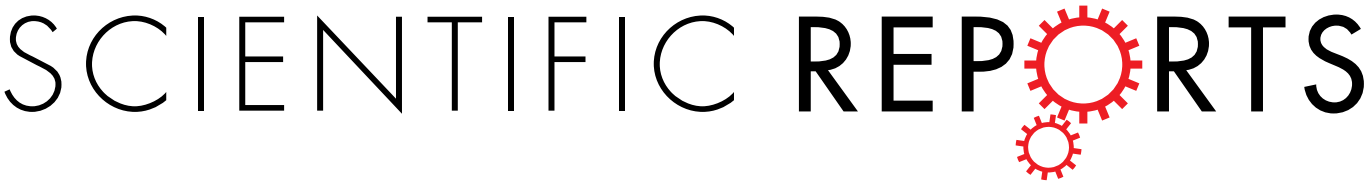
289. Ito, T. *et al.* Tissue Depletion of Taurine Accelerates Skeletal Muscle Senescence and Leads to Early Death in Mice. *PLoS One* **9,** e107409 (2014).

290. Kawai, M. *et al.* Nocturnin: a circadian target of Pparg-induced adipogenesis. *Ann. N. Y. Acad. Sci.* **1192,** 131–8 (2010).

291. Kawai, M. & Rosen, C. J. PPAry2: a circadian transcriptional factor in adipogenesis and osteogenesis. *Nat. Rev. Endocrinol.* **6,** 629–636 (2011).

292. Mortensen, O. H. 8. Taurine and metabolic disease 1. **661,** 167–190 (2012).

8. APPENDIX



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Taurine Treatment Modulates Circadian Rhythms in Mice Fed A High Fat Diet

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Close ties have been made among certain nutrients, obesity, type 2 diabetes and circadian clocks. Among nutrients, taurine has been documented as being effective against obesity and type 2 diabetes. However, the impact of taurine on circadian clocks has not been elucidated. We investigated whether taurine can modulate or correct disturbances in daily rhythms caused by a high-fat diet in mice. Male C57BL/6 mice were divided in four groups: control (C), control + taurine (C+T), high-fat diet (HFD) and HFD + taurine (HFD+T). They were administered 2% taurine in their drinking water for 10 weeks. Mice were euthanized at 6:00, 12:00, 18:00, and 24:00. HFD mice increased body weight, visceral fat and food intake, as well as higher levels of glucose, insulin and leptin, throughout the 24 h. Taurine prevented increments in food intake, body weight and visceral fat, improved glucose tolerance and insulin sensitivity and reduced disturbances in the 24 h patterns of plasma insulin and leptin. HFD downregulated the expression of clock genes *Rev-erbα*, *Bmal1*, and *Per1* in pancreatic islets. Taurine normalized the gene and protein expression of PER1 in beta-cells, which suggests that it could be beneficial for the correction of daily rhythms and the amelioration of obesity and diabetes.

Diverse physiological and behavioral circadian oscillations such as sleep-wake cycles and the secretion of hormones and metabolism are controlled by the molecular clock that generates daily rhythms in mammals. This clock allows for adaptation to periodic changes in the environment, according to light and dark cycles[1,2].

Circadian rhythms are controlled by cell-autonomous and self-sustained oscillators, which depend on the transcription-translation, autoregulatory feedback loop of specific clock genes. The positive limb is formed by transcription factors, including *Bmal1/Clock,* that activates the transcription of *Per and Cry* genes, which drive the negative limb and inhibit the activity of *Bmal1/Clock*, generating rhythmic oscillations of gene expression. An additional regulation is driven by the nuclear receptors *Reverb's* and *Ror's*, which repress and activate *Bmal1*, respectively[3–5]. Mammalian circadian clocks are composed of a central clock located in the suprachiasmatic nucleus (SCN) in the hypothalamus and several peripheral clocks found in many tissues; including the pancreas, the liver, adipose tissue and muscle[6–12]. Peripheral clocks contribute to global energy metabolism and participate in local metabolic functions, such as those involved in glucose and lipid homeostasis. Among peripheral clocks, the pancreatic clock is crucial for controlling the function of pancreatic beta-cells. Ablation of *Clock* and *Bmal1* with genetic manipulation led to the development of diabetes in mice, whereas *Rev-erbα* gene silencing led to impairments in beta- and alpha-cell function[8,10,11,13].

Other factors apart from genetic manipulation can also disrupt clock gene expression in peripheral tissues. For instance, nutritional insults such as HFD feeding disrupted the expression of *Rev-erbα*, *Clock*, *Per1*, and *Per2* in mouse pancreatic islets, followed by disruption of the pattern of insulin secretion[10]. In addition, nutrient signaling, by glucose or amino acids may act as a training agent of SCN and peripheral clocks, leading to tissue-specific differences in the expression of clock genes[14–16]. Dietary supplementation of the amino sulfonic acid taurine was found to improve whole body glucose control particularly, and to be useful in both prevention and treatment for metabolic complications in obese rodents[17,18]. In this point, taurine is considered an essential nutrient due

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to the diverse therapeutic effects at different levels and could be proposed as a supplement in obesity treatment. Otherwise important external stimuli, like nutrients, synchronize the circadian clock, however the effect of taurine on daily rhythms of hormones and on the expression of clock genes has never been studied before. Therefore, the aim of this study was to determine whether taurine treatment can modulate and prevent disturbances of daily rhythms of hormones and the expression of clock genes caused by HFD.

Results

Effects of taurine treatment on body weight, visceral fat and food intake. We first measured body weight progression from the first week of treatment. Body weight was similar between the groups until the 5th week of treatment. In the HFD group, body weight increased from the 5th week of treatment in both HFD and HFD+T treated mice, as compared to controls. However, from the 8th to the 10th week of treatment, mice fed with HFD+T prevented the increase in body weight, compared to mice fed with HFD until the end of treatment (Fig. 1A). After 10 weeks of treatment, visceral fat weight was comparable between the C and C+T groups. As expected, mice treated with a HFD showed a significant increase in visceral fat, compared to the C group, whereas mice treated with HFD+T had a decrease in visceral fat, compared to the HFD group (Fig. 1B). Interestingly, HFD-treated mice had increased food intake already at the first week of treatment with a peak of food consumption at the second week and a sustained elevation of food intake until the end of treatment. On the other hand, HFD+T mice had decreased food intake already at the first week compared to the HFD group (Fig.1C). Measurements of food intake at the end of treatment (10th week) during the light cycle was similar between C and C+T groups and increased during the dark cycle in both groups ($P < 0.0001$, respectively). In contrast, mice fed a HFD exhibited an increase in food intake during both light and dark cycles, as compared to C group (Fig. 1D). Strikingly, taurine decreased food intake in both light and dark cycles. Water intake was similar in all experimental groups at the 10th week of treatment (Fig. 1E). The effects of the HFD and taurine treatment after 10 weeks on body weight gain, visceral fat and food intake and their interactions are also shown in Table 1. There was no effect of taurine treatment on body weight, visceral fat and food intake in the control group (Table 1). However, the effect of HFD alone when compared to the control group showed that this diet increased body weight, visceral fat and food intake. On the other hand, taurine treatment in the HFD group was able to decrease body weight, visceral fat and food intake although the treatment could not decrease these parameters at the level of the control group (Table 1). To check the effect of taurine at the beginning of the treatment we next measured body weight, visceral fat, water and food intake at the first week of treatment. There was no difference in body weight, visceral fat and water intake in all experimental groups (Supplementary Fig. 1A–C, respectively). Strikingly, mice fed a HFD had an increase in food intake during the light and dark cycles already at the first week of treatment whereas taurine was able to prevent the increase in food intake caused by HFD in both light and dark cycles (Supplementary Fig. 1D). Moreover, one week of HFD treatment led to glucose intolerance (Supplementary Fig. 2A) and decreased insulin sensitivity (Supplementary Fig. 2C) but taurine treatment in mice fed a HFD had a small effect on glucose tolerance and insulin sensitivity with no statistically significant results when calculating the area under the curve (Supplementary Fig. 2B,D respectively). These results showed that one week of taurine treatment in mice treated with HFD can decrease food intake during light and dark cycles even before any change in body weight and visceral fat. In addition, prolonged taurine treatment can modulate the increase in body weight, visceral fat and food intake caused by HFD feeding.

Effects of taurine treatment on daily glucose, insulin and leptin levels. The effect of taurine treatment (C+T group) on the daily glucose levels was evident at time 12:00, with lower levels than in the C group (Fig. 2A). As expected, blood glucose levels were elevated at 18:00 and 24:00 in the HFD group, compared to the C group (Fig. 2A). However, mice treated with HFD+T exhibited lower glucose levels throughout the 24 h period as compared to mice treated only with HFD (Fig. 2A). Mesor analysis of the data confirmed the hypoglycemic effect of taurine during HFD treatment by decreasing glucose levels to the same levels of the control group (Table 2).

Plasma insulin levels were similar in the C group at all time points measured, but increased in the C+T group at 24:00 (Fig. 2B). In contrast, insulin levels were continuously elevated throughout the 24 h period in the HFD group as compared to the C group. Interestingly, HFD+T decreased the overall levels of plasma insulin throughout the 24 h period and increased plasma insulin levels at 24:00 (Fig. 2B).

Control mice exhibited statistically significant variations in plasma leptin concentrations with decreased values at 12:00, and a peak at 24:00 ($P < 0.01$ and $P < 0.05$). The C+T group exhibited the same variations in plasma leptin, but the peak occurred at 18:00 (Fig. 2C). Interestingly, HFD mice disrupted the daily pattern of leptin, with no decrease in leptin levels at 12:00 and a peak of leptin at 18:00 ($P < 0.05$). Taurine treatment prevented the disruption of daily plasma leptin caused by HFD, decreased leptin levels at 12:00 ($P < 0.01$) and a peak of leptin from 12:00 to 24:00 ($P < 0.01$). Confirming these results, the mesor and amplitude values showed that taurine decreased insulin and leptin levels in mice treated with a HFD (Table 2). Our results demonstrated that taurine supplementation in mice fed a HFD can restore the 24 h pattern of plasma leptin levels.

Effects of taurine on glucose tolerance and insulin sensitivity. Glucose tolerance was similar between C and C+T mice, showing no differences between the groups. As expected, HFD mice displayed impaired glucose tolerance, evident in the total area under the curve (AUC) as compared to control mice (Fig. 3A,B). Taurine supplementation in HFD-fed mice prevented glucose intolerance caused by a HFD, as indicated in the total area under the curve (AUC) (Fig. 3B). There were no differences in insulin sensitivity between C and C+T mice, but the HFD group exhibited impaired insulin sensitivity (Fig. 3C). Ten weeks of taurine treatment were sufficient to prevent insulin resistance caused by HFD feeding, as shown in the total area under the curve (AUC) (Fig. 3C,D). Plasma insulin levels during the ipGTT showed a significant decrease in plasma insulin levels in the C+T group at 15 min. In accordance with the ipGTT results, the HFD group displayed elevated

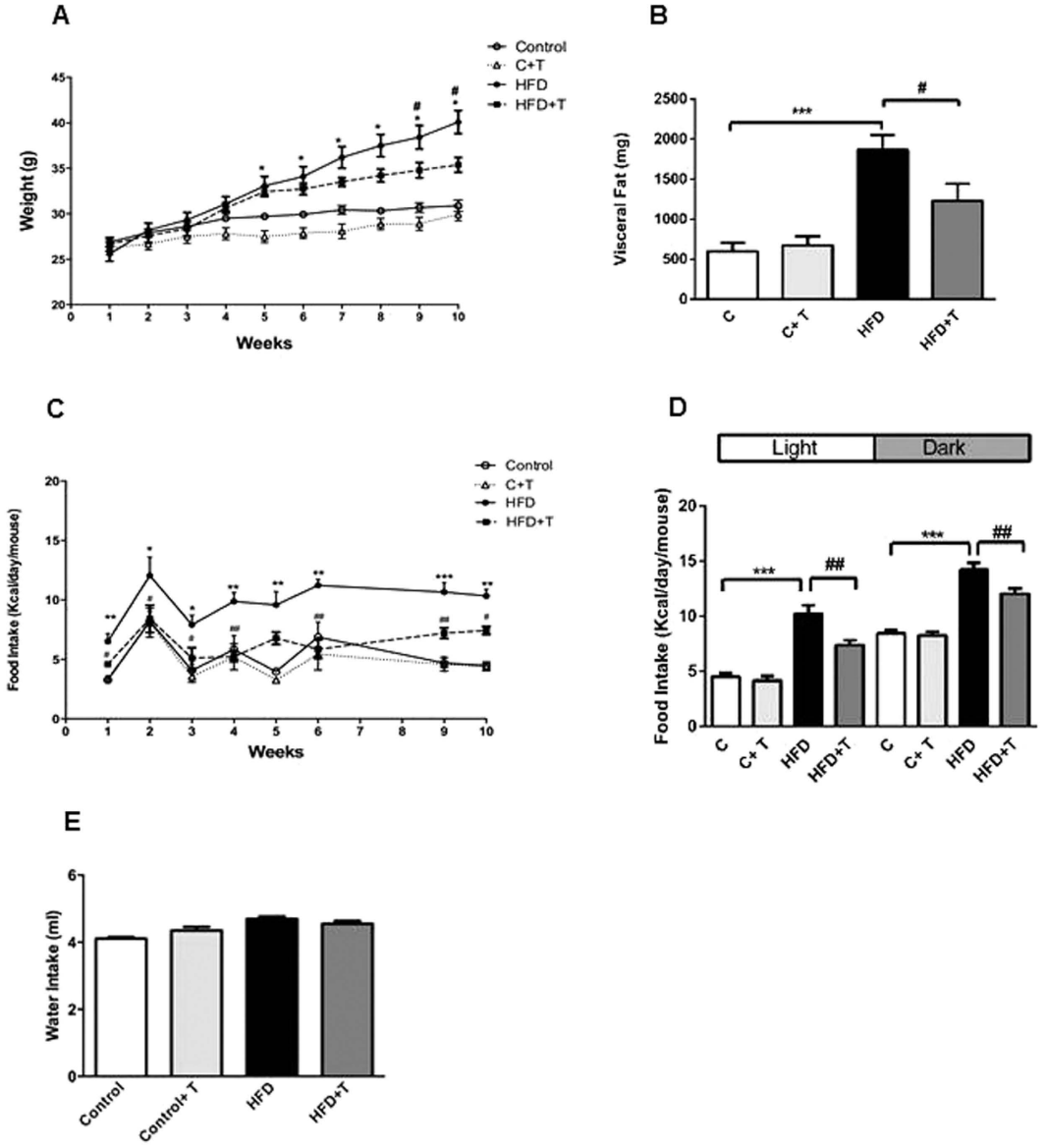

Figure 1. Effects of taurine treatment on body weight, visceral fat, food intake. Mice were treated with chow diet (C), Chow + Taurine (C + T), High-fat diet (HFD) or HFD + Taurine (HFD + T) for 10 weeks. (**A**) Body weight progression from the first day of taurine treatment until the 10th week of treatment in mice fed with chow or HFD. (n = 8–10 mice per group). Differences between C vs HFD (*$P < 0.05$); HFD vs HFD+T ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$). (**B**) Visceral fat weight after 10 weeks of taurine treatment in mice fed with chow or HFD. Differences between C vs HFD (*$P < 0.05$); HFD vs HFD+T ($^{\#}P < 0.05$). (n = 8–10 mice per group). (**C**) Food intake progression from the first week until the 10th week of treatment. Differences between C vs HFD *$P < 0.05$. **$P < 0.001$, ***$P < 0.0001$. Differences between HFD vs HFD+T ($^{\#}P < 0.05$, $^{\#\#}P < 0.001$). (**D**) Food intake at 10th week of treatment during the light and dark cycle. Food was measured by weighing the food consumption separately during the day (from 8:00 to 20:00) and the food consumption during the night (from 20:00 to 8:00). Differences between C vs HFD (***$P < 0.001$); and HFD vs HFD+T ($^{\#\#}P < 0.01$). (**E**) Water Intake at 10th week of treatment. Data are expressed as mean ± SEM.

insulin levels at 30 and 60 min, as compared to the C group. Interestingly, taurine treatment reduced insulin levels at 30 min and 60 min in animals treated with HFD (Fig. 3E), as indicated in the total area under the curve (AUC) (Fig. 3F).

Effects of taurine on daily insulin secretion *in vitro*. To check whether the modulation of taurine on the 24 h pattern of insulin secretion *in vivo* reflects changes in insulin secretion *in vitro*, we next performed glucose-stimulated insulin secretion from fresh isolated islets at 6:00, 12:00, 18:00, and 24:00. Isolated islets from control mice exhibited no changes in insulin secretion during the 24 h period at low glucose concentrations. Islets from the C+T group had increased insulin secretion at 18:00 when stimulated with basal glucose concentrations (Fig. 4A). When control islets were stimulated with high glucose concentrations, we could observe the

Variable	Groups				p Value interactions		
	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vs HFD+T
Body weight gain (g)	4 ± 0,8	3,7 ± 0,5	14,5 ± 0,9***	8,7 ± 0,8##	0,76	0,001	0,01
Visceral fat (mg)	596,4 ± 109,7	673,5 ± 112,6	1866,3± 183,5***	1229,8 ± 214,7#	0,63	0,001	0,03
Final Food intake (kcal/day/mouse)	6,4 ± 0,3	5,9 ± 0,3	11,05 ± 0,4***	8,5 ± 0,4#	0,32	0,001	0,05
Differences between groups							
C vs C+T ns (not significant)							
C vs HFD	***p < 0.05						
HFD vs HFD+T	#p < 0.05						
	##p < 0.01						

Table 1. Effects of taurine treatment on body weight, visceral fat and food intake. Effect of HFD and taurine after 10 weeks of treatment. Values shown are the means ± SEM. (Two-way ANOVA followed by Bonferroni post hoc test).

effect of the time of the day on insulin secretion. Glucose-stimulated insulin secretion had a peak of secretion at 18:00 and maintained high levels at 24:00 as compared to secretion at 6:00 and 12:00. The same pattern of glucose-stimulated insulin secretion was observed in islets from the C+T group, however, taurine treatment augmented glucose-stimulated insulin secretion during the dark cycle, as compared to secretion from control islets (Fig. 4A). When islets from HFD mice were stimulated with high glucose concentrations, we found alterations in insulin secretion according to the time of day. Islets isolated at 6:00 lost the stimulatory effect of glucose compared to control islets (Fig. 4B). The nocturnal increase of glucose-stimulated insulin secretion was advanced to 12:00, exhibiting an increase in insulin secretion already at 12:00 and keeping high levels throughout the dark cycle. We could not detect the effect of the time of day in isolated islets from mice treated with HFD+T on glucose-stimulated insulin secretion. However, islets from HFD+T decreased glucose-induced insulin secretion at 12:00, 18:00 and 24:00, as compared to the HFD group (Fig. 4B). These results indicate that HFD disrupts *in vitro* glucose-stimulated insulin secretion that could not be prevented by taurine treatment.

Effects of taurine on the clock gene expression in pancreatic islets. The islet exhibits oscillations in clock gene expression throughout the day and alterations in clock gene expression impaired beta-cell function leading to the development of diabetes[8–10,19]. Therefore, we next checked whether taurine could modulate the daily pattern of *Bmal1*, *Clock*, *Rev-erbα*, *Per1* and *Per2* expression in isolated pancreatic islets. The expression of *Clock* was similar in control or C+T islets (Fig. 5A). There was no effect of HFD on *Clock* expression, but taurine treatment in the HFD group increased *Clock* expression at 18:00 (Fig. 5A). This effect was evident by the mesor and amplitude values showing an increase in the HFD+T group (Table 3). The 24 h pattern of *Bmal1* in control islets showed the highest peak at 6:00 and the lowest at 18:00 (Fig. 5B). Taurine treatment in mice fed with chow diet showed an increase in mesor values detected by the cosinor analysis. Islets from HFD downregulated *Bmal1* expression at 6:00, as compared to control islets, whereas HFD+T islets exhibited increased *Bmal1* expression at 24:00 (Fig. 5B). Cosinor analysis showed that HFD feeding decreased the mesor and amplitude of *Bmal1* expression and that taurine treatment restored mesor values of *Bmal1* expression and changed the acrophase (from 6 in all groups to 23 h) in mice treated with HFD (Table 3). *Rev-erbα* expression showed a similar pattern of expression in C and C+T islets, with a peak of expression at 12:00 and 18:00 and a decrease at 6:00 and 24:00 (Fig. 5C). HFD disrupted 24 h expression by upregulating *Rev-erbα* expression at 6:00 and downregulating at 18:00, compared to C islets, while there was no effect of taurine in HFD+T islets on *Rev-erbα* expression (Fig. 5C). Cosinor analysis confirmed this data, showing a decrease in the values of the mesor in the HFD group, in relation to the C group (Table 3). *Per2* expression had a peak of expression at 18:00 in control islets in all experimental groups, with increased *Per2* levels in the C+T group at 12:00 compared to control. There was no effect of diet and taurine among the other groups (Fig. 5D). In C and C+T islets, *Per1* showed changes during the 24 h pattern of expression with the highest peak at 18:00 (Fig. 5E). HFD downregulated *Per1* expression at 6:00 and at 18:00, as compared to C islets. Interestingly, taurine treatment during HFD feeding restored the circadian pattern of *Per1* expression to the same expression levels as in the C islets (Fig. 5E). Cosinor analysis confirmed the inhibitory effect of HFD on *Per1* expression and in the amplitude of the gene, as well as the preventive effect of taurine on the *Per 1* expression and *Per1* amplitude (Table 3). These results demonstrated that taurine treatment during HFD feeding can prevent the disruption of *Per1* expression in pancreatic islets.

PER1 protein expression in pancreatic islets. Since taurine modulated the expression of *Per1* in islets, we next quantified protein levels of PER1 in pancreatic islets isolated at 18:00 and 24:00 after 10 weeks of treatment. Confirming our results, the HFD decreased the protein expression of PER1 at 18:00, and taurine restored PER1 protein levels in islets from mice treated with a HFD (Fig. 6A). As expected, there was no difference in PER1 protein expression at 24:00, confirming our results on gene expression ($P = 0.46$).

To further validate these results, immunofluorescence was performed in whole fresh isolated pancreatic islets of mice after 10 weeks of treatment. We analyzed total percentage of PER1-positive stained cells within the islet and found a higher tendency for PER1 staining in C+T islets ($P = 0.09$), as compared to control islets (Fig. 6B). Moreover, C+T islets showed a statistically significant increase in the percentage of total PER1 in insulin-positive cells when compared with C islets (Fig. 6B). Since PER1 is a transcription factor that can be localized in the

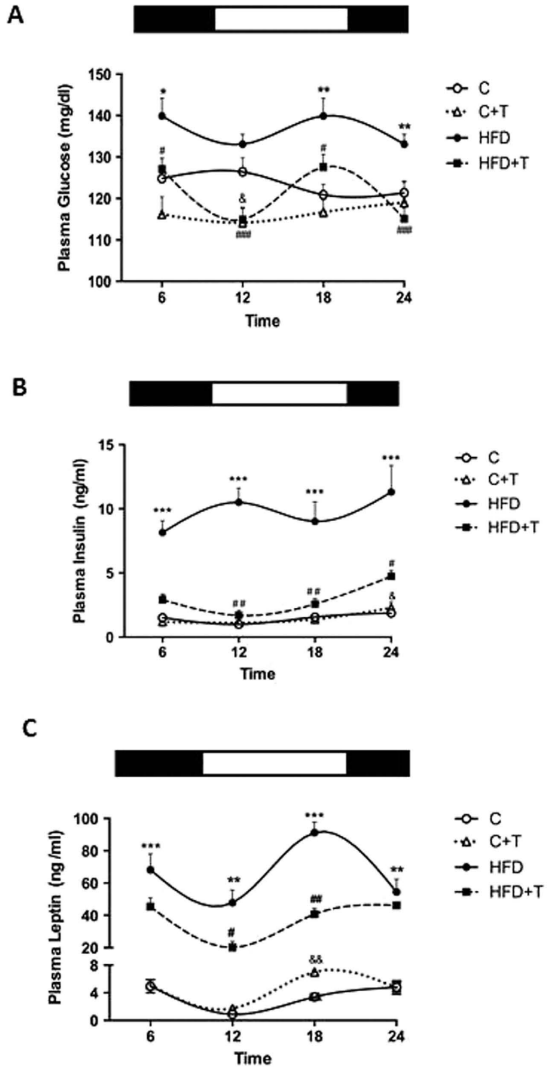

Figure 2. Effects of taurine treatment on daily glucose, insulin and leptin levels. (**A**) 24h time course measurements of plasma glucose concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+T ($^{\&}P < 0.05$). Differences between C vs HFD ($^{*}P < 0.05$, $^{**}P < 0.001$). Differences between HFD vs HFD+T ($^{\#}P < 0.05$ $^{\#\#\#}P < 0.001$). (n = 8–10 mice per group). (**B**) 24 h time course measurements of plasma insulin concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD. (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+T ($^{\&}P < 0.05$). Differences between C vs HFD ($^{***}P < 0.001$). Differences between HFD vs HFD+T ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$) (n = 8–10 mice per group). (**C**) 24 h time course measurements of plasma leptin concentrations after 10 weeks of taurine treatment in mice fed a chow diet or HFD (C), (C+T) (HFD) and (HFD+T). Differences between C vs C+T ($^{\&\&}P < 0.01$). Differences between C vs HFD ($^{**}P < 0.05$, $^{***}P < 0.001$). Differences between HFD vs HFD+T ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$). (n = 8–10 mice per group). The black bars refers on the top of the figures to the dark cycle and the white bars to the light cycle.

cytoplasm and/or nucleus, we accessed the cell localization of PER1 in isolated islets. We found similar nuclear PER1 localization in the beta-cells of the C and C+T groups (Fig. 6B). However, when measuring nuclear staining in all islet cells, PER1 expression was upregulated by taurine in the control group (Fig. 6B).

Consistent with our results on gene expression, the HFD downregulated PER 1 protein expression as compared to C islets ($P = 0.002$). When we compared islets from HFD and HFD+T, we found an increase in total PER1-positive staining with taurine (Fig. 6C). Moreover, insulin-positive cells from HFD+T islets presented higher total PER1 labelling than islets from HFD mice (Fig. 6C). We observed an increased staining of nuclear PER1 from HFD+T islets and nuclear PER1 staining when analyzed in total islet cells, compared to islets from HDF mice (Fig. 6C). Thus, these results demonstrated that HFD downregulated PER1 protein and gene expression, whereas taurine upregulated PER1 in mice fed a HFD.

	R^2				Mesor				p Value			Amplitude				p Value			Acrophase				p Value		
	C	C+T	HFD	HFD+T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vs HFD-T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vs HFD-T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vs HFD-T
Glucose	0,028	0,02	0,001	0,003	123,7	$116^{\&\&}$	$136,5^{**}$	$121,17^{\#\#\#}$	0,01	0,005	0,001	11,6	12,9	12,1	9,4	0,76	0.85	0.25	11,0	$23,0^{\&\&\&}$	17,0	17,0	0,001	0,96	0,9
Insulin	0,24	0,38	0,01	0,60	1,4	1,5	$9,3^{***}$	$2,9^{\#\#}$	0,87	0,001	0,007	0,6	0,7	$3,3^{***}$	$1,8^{\#}$	0,7	0.001	0.04	23,0	23,0	17,0	23,0	0,77	0,35	0,9
Leptin	0,49	0,34	0,06	0,48	3,5	4,6	$64,3^{***}$	$38,9^{\#\#}$	0,11	0,001	0,007	2,6	2,5	$27,4^{***}$	$15,8^{\#\#}$	0,61	0.001	0.01	23,0	23,0	17,0	23,0	0,32	0,68	0,2
Differences between groups																									
C vs C+T	$^{\&\&}p < 0.01$																								
	$^{\&\&\&}p < 0.001$																								
C vs HFD	$^{**}p < 0.01$																								
	$^{***}p < 0.001$																								
HFD vs HFD+T	$^{\#}p < 0.05$																								
	$^{\#\#}p < 0.01$																								
	$^{\#\#\#}p < 0.001$																								

Table 2. Cosinor analysis of the 24 h expression of metabolic parameters. Cosinor analysis including goodness of the fit ($\mathbf{R}^2$), mesor, amplitude, and acrophase of the 24 h profiles of plasma glucose, insulin and leptin. Next Two-way ANOVA shows the difference between groups in mesor, amplitude and acrophase respectively. Values shown are the means ± SEM.

Effects of taurine on the clock gene expression on visceral adipose tissue. We next checked whether taurine could modulate the daily pattern of *Bmal1*, *Clock*, *Rev-erbα*, *Per1* and *Per2* expression in visceral adipose tissue. The expression of *Clock* was similar in control or C+T islets (Supplementary Fig. 3A). However, HFD disrupted *Clock* expression in visceral adipose tissue by increasing *Clock* expression at 6:00 and at 18 and 24:00. Taurine treatment was able to restore the *Clock* expression at 18:00 and 24:00 in mice fed with HFD (Supplementary Fig. 3A). *Bmal1* expression in control group had a peak of expression at 6:00 and 24:00, and was decreased in C+T group at 6:00 and 24:00. *Bmal1* expression was downregulated by HFD at 12:00 and 24:00 but taurine treatment had no effect on *Bmal1* expression (Supplementary Fig. 3B). The expression of *Rev-erbα* in visceral adipose tissue was decreased at 6:00 and increased at 24:00 by HFD (Supplementary Fig. 3C) and taurine treatment could normalize *Rev-erbα* mRNA levels at 6:00 and 24:00. *Per1* expression was also decreased at 6:00 in visceral adipose tissue from mice fed a HFD but in this case, taurine treatment had no effect on *Per1* expression in visceral adipose tissue (Supplementary Fig. 3D). *Per 2* expression was increased in C+T group at 24:00 compared to control. In addition, HFD disrupted *Per2* expression by upregulating the expression of this gene at 6:00 and 24:00 whereas taurine treatment restored *Per2* expression at both 6 and 24:00 in mice treated with HFD (Supplementary Fig. 3E). Thus, taurine treatment during HFD feeding can prevent the disruption of *Clock*, *Rev-erbα* and *Per2* expression in visceral adipose tissue.

Discussion

Close ties are found among nutrients, metabolic diseases and circadian clocks. This paper is the first to study the modulation of circadian rhythms by taurine. Taurine is one of the most abundant sulphonic acids in all tissues, and the efficacy of taurine administration against obesity and type 2 diabetes has been well documented. However, the impact of taurine on circadian clocks has not been studied before. In our results, HFD treatment significantly increased body weight and visceral fat and decreased glucose tolerance and insulin sensitivity, as previously established in other studies[10,20,21]. Taurine treatment prevents increases in body weight, glucose intolerance, insulin resistance and reduced visceral fat, according to studies where taurine had several beneficial metabolic effects in different models of type 2 diabetes and obesity[22–26].

It has been demonstrated that consumption of a HFD disrupts the 24 h pattern of circulating levels of several hormones and the behavioural and molecular circadian rhythms in rodents[10,27,28]. We found that HFD increments food intake already at the first week of treatment during the light and dark cycles and that taurine treatment prevents HFD-increased food intake during both resting and active periods at the first and at 10th week of treatment. Since the effect on food intake occurs already at the first week of treatment before any changes in body weight and fat weight this, suggest an early a central effect of taurine on food intake. Indeed, this effect could be explained by a direct effect of taurine on the hypothalamic regions that control food intake. Taurine has an anorexigenic action in the hypothalamus and modulates hypothalamic neuropeptide expression[29,30]. Another possibility could be a direct effect of taurine on the secretion of hormones that regulate food intake, including insulin and leptin. Indeed, we demonstrate that taurine treatment in mice fed with a HFD decreased the overall levels of plasma insulin during 24 h; it also decreased the insulin levels during the glucose tolerance test and diminished glucose stimulated insulin secretion in isolated islets. The 24 h pattern of plasma leptin levels was also reduced in mice treated with HFD+T, which could be explained by the decrease in visceral fat in these animals.

Diurnal variations in glucose, leptin and plasma insulin levels are subjected to daily cycles, which are altered in diabetes and obesity[10,31–37]. Our results confirm the disruption of leptin levels by HFD feeding and provide new insights regarding the preventive effect of taurine on leptin levels in animals fed a HFD. The mechanisms underlying the preventive effects of taurine on leptin levels are not yet known, but it is possible that taurine, by regulating insulin levels, could normalize the 24 h pattern leptin levels. In fact, daily leptin levels were shown to be dependent on insulin levels in mice[38].

The daily pattern of insulin was shown to be independent of the temporal distribution of feeding in rats[34], and an intrinsic circadian oscillator composed of clock and clock-controlled genes was found in mice,

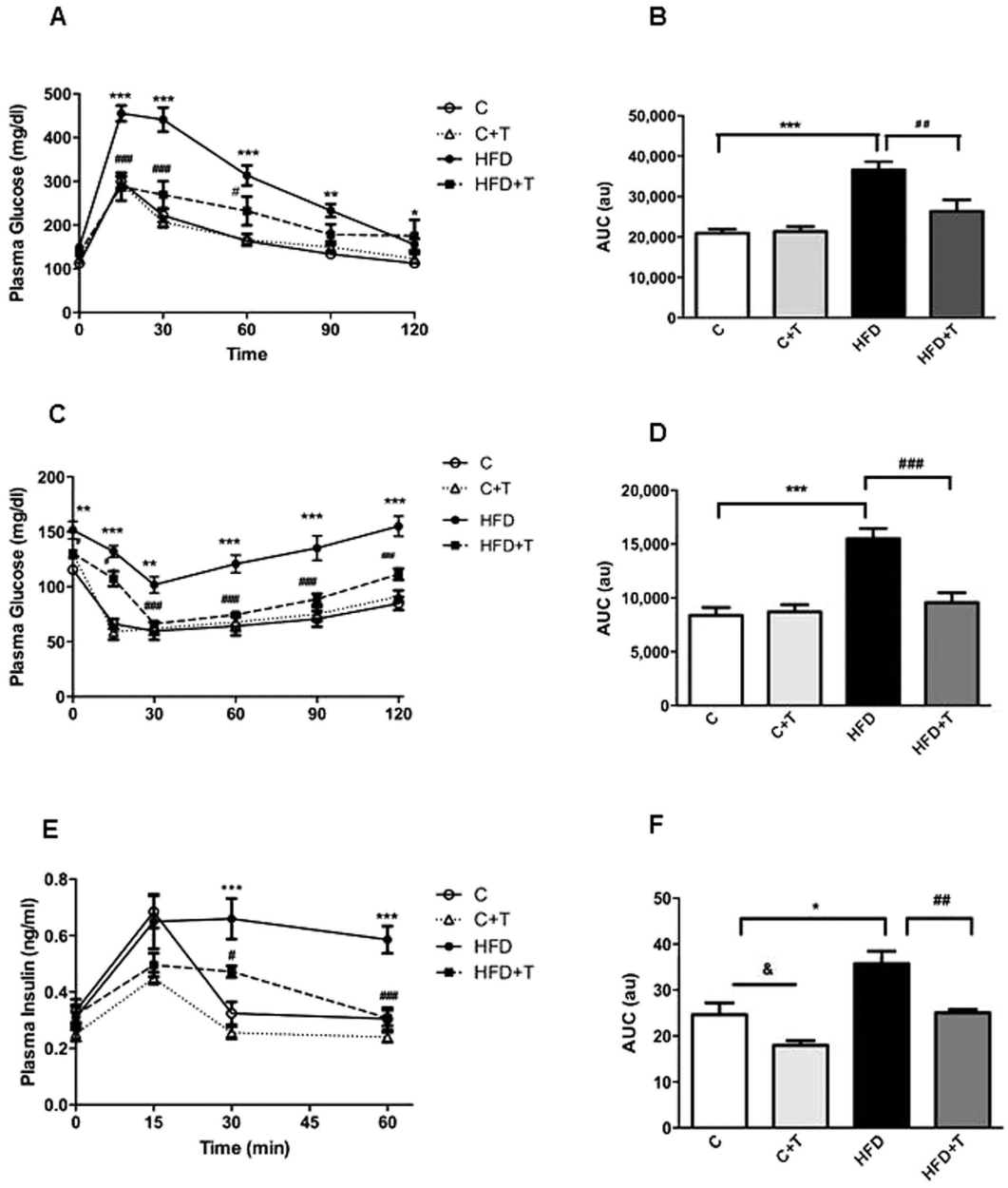

Figure 3. Effects of taurine treatment on glucose tolerance and insulin sensitivity. Glucose tolerance test (**A**) (C), (C+T), (HFD) and (HFD+T). Differences between C vs HFD (*P < 0.05, **P < 0.01 ***P < 0.001); HFD vs HFD+T ($^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001). (n = 8–10 mice per group). (**B**) Area under the curve (AUC). Differences between C vs HFD (***P < 0.001) and HFD vs HFD+T (**P < 0.01). (**C**) Insulin tolerance test (n = 8–10). Differences between C vs HFD (**P < 0.01 ***P < 0.001); HFD vs HFD+T ($^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001). (**D**) Area under the curve (AUC), (***P < 0.001). (**E**) Plasma insulin measured during the glucose tolerance test at 0, 15, 30, and 60 min (n = 5). Differences between C vs HFD (***P < 0.001); HFD vs HFD+T ($^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001) and C vs C+T ($^{\&\&}$P < 0.01). (n = 8–10 mice per group).

rats and humans[8–10,39,40]. Although we could not detect a 24 h pattern of insulin levels in our *in vivo* results, glucose-stimulated insulin secretion in isolated islets changed according to the time of day. Insulin secretion in control islets stimulated with glucose were elevated during the active period and reduced during the resting period, with enhanced circadian insulin secretion in mice fed a chow diet and treated with taurine. The 24 h pattern of glucose-stimulated insulin secretion was altered in islets from HFD mice, which showed a peak in insulin secretion during the resting period, consistent with the increase in food intake during the light cycle in these mice. Surprisingly, taurine treatment abolished the daily pattern of glucose-stimulated insulin secretion in isolated islets from HFD-fed mice. The reason is not known, but it could be possible that taurine could modulate important metabolic genes involved in the regulation of insulin secretion and beta-cell mass, as shown in previous studies with taurine supplementation in obese animals[41,42].

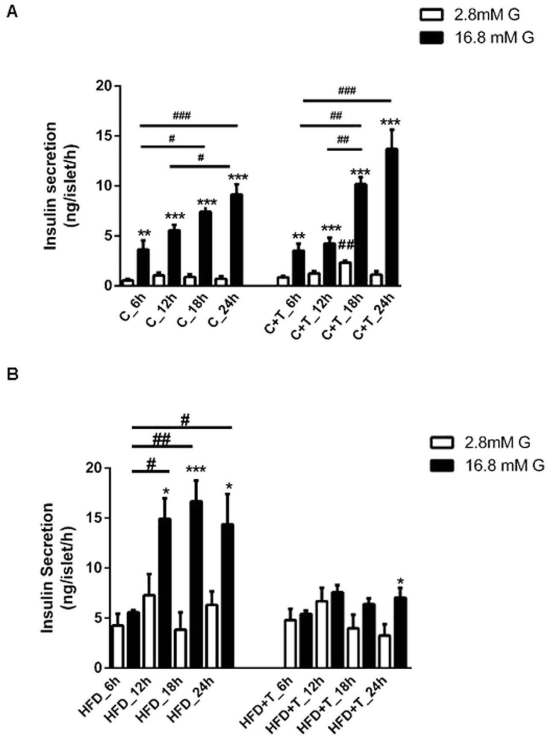

Figure 4. Effects of taurine on circadian insulin secretion *in vitro*. Pancreatic islets were isolated at different times of day (6:00 h, 12:00 h, 18:00 h and 24:00 h) after 10 weeks of taurine treatment and stimulated with 2.8 mM glucose (white bars) and 16.8 mM glucose (black bars) (n = 6–7 mice per group). (**A**) Insulin secretion from C and C+T groups. Data are expressed as mean ± SEM. Differences between 2.8 mM glucose and 16.8 mM glucose in the C (**$P < 0.01$, ***$P < 0.001$) and C+T groups (**$P < 0.01$, ***$P < 0.001$). Differences in the time of day at 2.8 mM glucose and 16.8 mM glucose in the C and C+T groups ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ respectively). (**B**) Insulin secretion from HFD and HFD+T groups (n = 6–7). Differences between 2.8 mM glucose and 16.8 mM glucose in the HFD group (*$P < 0.05$, ***$P < 0.001$) and HFD+T group (*$P < 0.05$). Differences in the time of day at 16.8 mM glucose in HFD group ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$).

The islet clock exhibits oscillations in clock gene expression throughout the day in different species, and alterations in their expression leads to impairments in insulin secretion[8,10,13,43,44]. Our results show that the HFD disrupts the expression of *Bmal1*, *Rev-erb alpha* and *Per1* genes in isolated islets, whereas taurine supplementation could not rescue *Bmal1* and *Rev-erbα* expression. An interesting finding of the present study is that taurine could prevent the downregulation of *Per1* levels caused by a HFD in isolated islets. This was evident in measurements of gene and protein expression, mainly in the insulin-positive cells. The increase in percentage of PER1 expression in insulin-positive cells was detected in the nuclear fraction of these cells, suggesting that PER1 could be a target of taurine. The role of PER1 in the regulation of insulin secretion has never been demonstrated, but it could be possible that taurine modulation of PER1 expression could regulate insulin secretion.

Adipose tissue clocks have important role in maintenance of lipid homeostasis and are related with features of obesity. The expression of clock genes in visceral adipose tissue were altered during HFD feeding. Similar peaks according to the diurnal expression of *Bmal1*, *Rev-erbα* and *Per 1*, *Per 2* during HFD were comparable with previous works respectively[28,45]. Taurine changed the expression of *Clock*, *Rev-erbα* and *Per2,* these effects of taurine could be tissue-dependent and peripheral clocks may be possible targets of this sulfonic acid during obesity.

Considering the different effects of *in vivo* taurine treatment on whole body metabolism, it is difficult that one mechanism could explain the taurine effects found in this study. However, it is clear from the present study that taurine improves disturbances in the 24 h pattern of plasma insulin and leptin, as well as *Per1* expression in pancreatic islets caused by HFD feeding. Thus, this is the first evidence that shows that taurine could be a potential target to correct or ameliorate the disturbances in circadian rhythms caused by obesity.

Materials and Methods

Animals and experimental groups. Ten-week-old male C57BL/6 mice were fed ad libitum with chow diet or high fat diet (45% fat Research diets Inc. D12451) for 10 weeks. The experimental groups were divided: Controls fed with chow diet (C), controls fed with chow diet and 2% taurine (Sigma-Aldrich, St. Louis, MO) in drinking water (C+T), mice fed with HFD (HFD) and mice fed with HFD and 2% taurine in water (HFD+T). Mice were allowed free access to food and water and maintained a 12 h light–dark cycle at 24 °C and constant humidity in soundproof cages. Body weight and water intake were measured every week during the 10 weeks of diet. Food intake was monitored from the first week of treatment by measuring the amount of food consumed from 8:00 to 20:00 (light cycle) and from 20:00 to 8:00 (dark cycle). After 10 weeks of treatment, mice

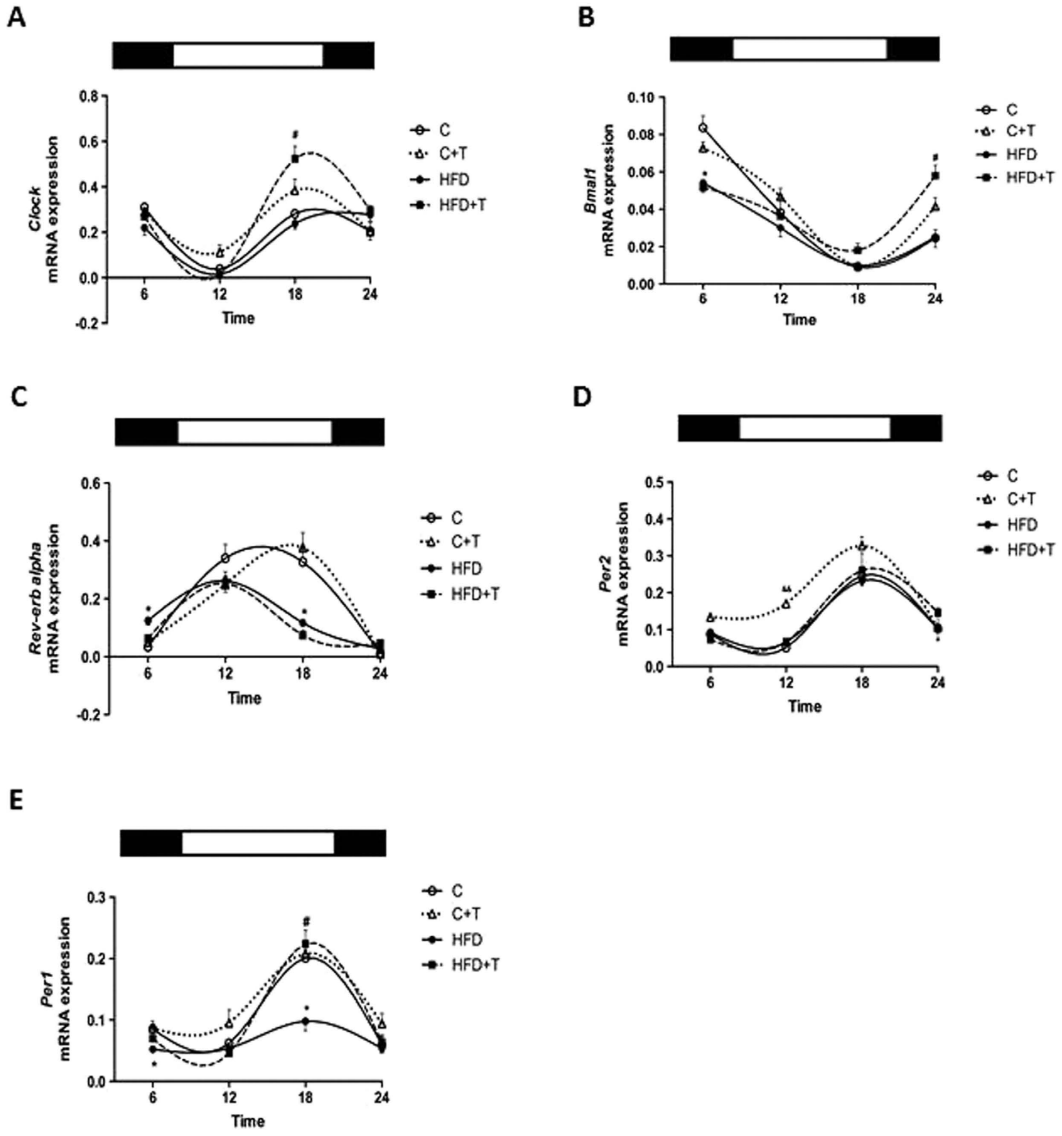

Figure 5. Clock genes expression in islets during 24 h. Pancreatic islets were isolated at different times of day (6:00 h, 12:00 h, 18:00 h and 24:00 h) after 10 weeks of taurine treatment. (n = 4–5). (**A**) *Clock* gene expression in isolated islets. (C), (C+T), (HFD) and (HFD+T). (**B**) *Rev-erb alpha* gene expression in islets. (**C**) *Bmal1* gene expression in islets. (**D**) *Per2* gene expression in islets. (**E**) *Per1* gene expression in islets. Data are expressed as mean ± SEM. Differences between C vs C+T ($^{\&\&}P < 0.01$), C vs HFD ($^{*}P < 0.05$) and HFD vs HFD+T ($^{\#}P < 0.05$) (n = 6–7 mice per group). The black bars refer on the top of the figures to the dark cycle and the white bars to the light cycle.

were euthanized at different times of day: 6:00, 12:00, 18:00 and 24:00 h. Protocols were approved by the Animal Research Committee of the University of Barcelona, Spain, and principles of laboratory animal care were followed, according to European and local government guidelines.

Daily measurements of glucose, insulin and leptin. The measurements of daily pattern of glucose, insulin and leptin were done in the fed state at the end of the long-term treatment of 10 weeks. Animals were sacrificed at the following times 6:00, 12:00, 18:00 and 24:00 and blood samples were collected to measure plasma insulin and leptin by ELISA (Mercodia Insulin, Uppsala Sweden and Crystal Chem, Harris County, TX, USA, respectively). Blood glucose was measured with glucometer (Accu-Check; Roche Diagnostics, Madrid, Spain).

Glucose and insulin tolerance tests. Glucose tolerance and insulin sensitivity were assessed during the last week of treatment. In the intraperitoneal glucose tolerance tests, 4 h-fasted mice were injected with 2 g glucose/kg body weight; blood glucose levels were then measured at 0, 15, 30, 60, 90 and 120 min after injection, and glucose was measured at all points using a glucometer (Accu-Check; Roche Diagnostics, Madrid, Spain). Moreover, blood samples were collected at the first 4 time points to quantify insulin. For insulin tolerance tests, mice were injected with 0.50 IU insulin/kg body weight. Blood samples were collected before insulin injection

	R^2				Mesor				p Value			Amplitude				p Value			Acrophase				p Value	
	C	C+T	HFD	HFD+T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vsHFD+T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD	HFD vs HFD+T	C	C+T	HFD	HFD+T	C vs C+T	C vs HFD
Rev-erb α	0,74	0,82	0,43	0,42	0,17	0,17	0,11*	0,08	0,91	0,05	0,25	0,17	0,18	0,11	0,09	0,67	0.17	0.55	11,0	17,0$^{\&}$	11,0	11,0	0,05	0,34
Clock	0,23	0,08	0,46	0,30	0,17	0,24	0,18	0,26$^{\#}$	0,13	0,63	0,05	0,16	0,23	0,16	0,28$^{\#}$	0,21	0.89	0.05	23,0	18,0	23,0	18,0	0,91	0,87
Bmal1	0,80	0,65	0,39	0,54	0,04	0,47$^{\&\&\&}$	0,03**	0,04$^{\#}$	0,001	0,01	0,02	0,04	0,04	0,02*	0,03	0,99	0.02	0.74	6,0	6,0	6,0	23,0$^{\&}$	0,57	0,22
Per1	0,46	0,42	0,36	0,60	0,10	0,12	0,06*	0,10$^{\#}$	0,12	0,05	0,05	0,07	0,09	0,03*	0,09$^{\#\#}$	0,20	0,04	0,01	17,0	17,0	18,0	18,0	0,40	0,42
Per2	0,41	0,37	0,46	0,36	0,12	0,20$^{\&\&}$	0,12	0,12	0,01	0,85	0,90	0,09	0,13	0,08	0,10	0,31	0,50	0,63	17,0	17,0	19,0	19,0	0,20	0,34
Differences between groups.																								
C vs C+T	$^{\&}p < 0.05$																							
	$^{\&\&}p < 0.01$																							
	$^{\&\&\&}p < 0.01$																							
C vs HFD	$^{*}p < 0.05$																							
	$^{**}p < 0.01$																							
HFD vs HFD+T	$^{\#}p < 0.05$																							
	$^{\#\#}p < 0.01$																							

Table 3. Cosinor analysis of the 24 h expression of clock genes. Cosinor analysis including goodness of the fit ($\mathbf{R^2}$), mesor, amplitude, and acrophase of the 24 h profiles of clock genes in pancreatic islets. Next Two-way ANOVA shows the difference between groups in mesor, amplitude and acrophase respectively. Values shown are the means ± SEM.

(time 0) and at 15, 30, 60, 90 min and 120 min after insulin administration, for glucose measurements. Plasma was separated by centrifugation. Plasma levels of insulin and leptin were measured by ELISA (Mercodia Insulin, Uppsala Sweden, and Crystal Chem, Harris County, TX, USA, respectively).

Isolation of pancreatic islets. After being fully anesthetized, mice were euthanized by cervical dislocation and a collagenase P solution (Sigma-Aldrich, St. Louis, MO, USA) was perfused into the pancreas through a direct puncture of the common bile duct to digest the pancreas. After digestion, islets of Langerhans were separated from exocrine tissue using a density gradient with Histopaque (Sigma-Aldrich, St. Louis, MO, USA). Islets were handpicked in a Leica stereomicroscope for further measurements of gene and protein expression and for insulin secretion assays.

Glucose-stimulated insulin secretion *in vitro*. After 10 weeks of treatment, mice were euthanized at 6:00, 12:00, 18:00 and 24:00. Groups of 5 fresh isolated islets from 4–5 different mice from each group were first pre-incubated at 37 °C in a 5.6 mM glucose KRBH solution for 30 min. Supernatant was discarded, and islets were incubated for 60 min at 37 °C in KRBH containing 2.8 mM or 16.7 mM glucose, respectively. After incubation, supernatants were collected, and insulin was quantified using a mouse insulin ELISA kit (Mercodia Insulin Uppsala, Sweden).

Western Blot. Protein extraction of isolated islets was obtained using RIPA lysis buffer (Tris 50 mmol/l, pH 7.5, EDTA 5 mmol/l, NaCl, 1% 150 mmol/l, Triton X-100 1%, SDS 0.1%, 10 mmol/l sodium fluoride, 1% sodium deoxycholate, and protease inhibitors). For the extraction, islet lysates were frozen and thawed twice after centrifugation for 20 min at 4 °C, and supernatants were collected and stored at −80 °C. Protein quantification was determined with the Lowry protein assay kit (Bio-rad, Hercules, CA, USA). The protein was electrotransferred onto a PVDF membrane. The membranes were blocked for 1 h with 0.05% Tween-20 and 5% NFDM, and then incubated overnight at 4 °C with the antibodies: AntiPer1 (1:500; Thermo Scientific Inc.) ß-Actin was used as a loading control (1:5,000; GE Healthcare, Hertfordshire, UK). Protein bands were revealed by using the Pierce ECL western blot substrate (Thermo Fisher Scientific, Madrid, Spain). Respective bands were quantified by densitometry. Image J software 1.50a and intensity values for PER 1 were normalized with ß-Actin.

RNA isolation and Real-time PCR. Total RNA was prepared from isolated islets using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was quantified using a Nanodrop 1000 (Thermo Scientific Wilmington, MA) and then retrotranscribed using the High Capacity cDNA Reverse Transcription Kit (AB Applied Biosystems, USA) following the manufacturer's instructions. The cDNA was amplified by Real-Time PCR in a LightCycler 480 System (Roche) using Mesa Green qPCR Master Mix (Mesa Green, Eurogentec, Belgium). The expression of clock genes in isolated pancreatic islets was measured, with the housekeeping gene *36B4* (*ribosomal protein large P0*) used as the endogenous control for quantification. The results were expressed as the relative expression with respect to control levels ($2^{-\Delta\Delta ct}$). The primer sequences are shown in Table 4.

Whole islet immunofluorescence. Fresh isolated islets were washed in buffer with PBS 1x and Triton 0.2%, then fixed in 4% of Paraformaldehide (Electron Microscopy Sciences, Hatfield, PA, USA) for 20 min, permeabilized with PBS with Triton 0.3% and blocked in a PBS solution with Triton 0.5% and FBS 10% for 1 h, and incubated overnight with the following primary antibodies: guinea pig anti-insulin 1:500, (Dako, Glostrup, Denmark) and anti-Per1 1:100 (Thermo Scientific, CA). Islets were incubated 2 hours with Secondary antibodies Cy3-labeled anti-guinea pig (Jackson Immuno research) and Alexa Fluor 488 (anti-rabbit (Molecular Probes, USA) were used at 1:200 dilutions, respectively. Nuclear staining was performed by using mounting media

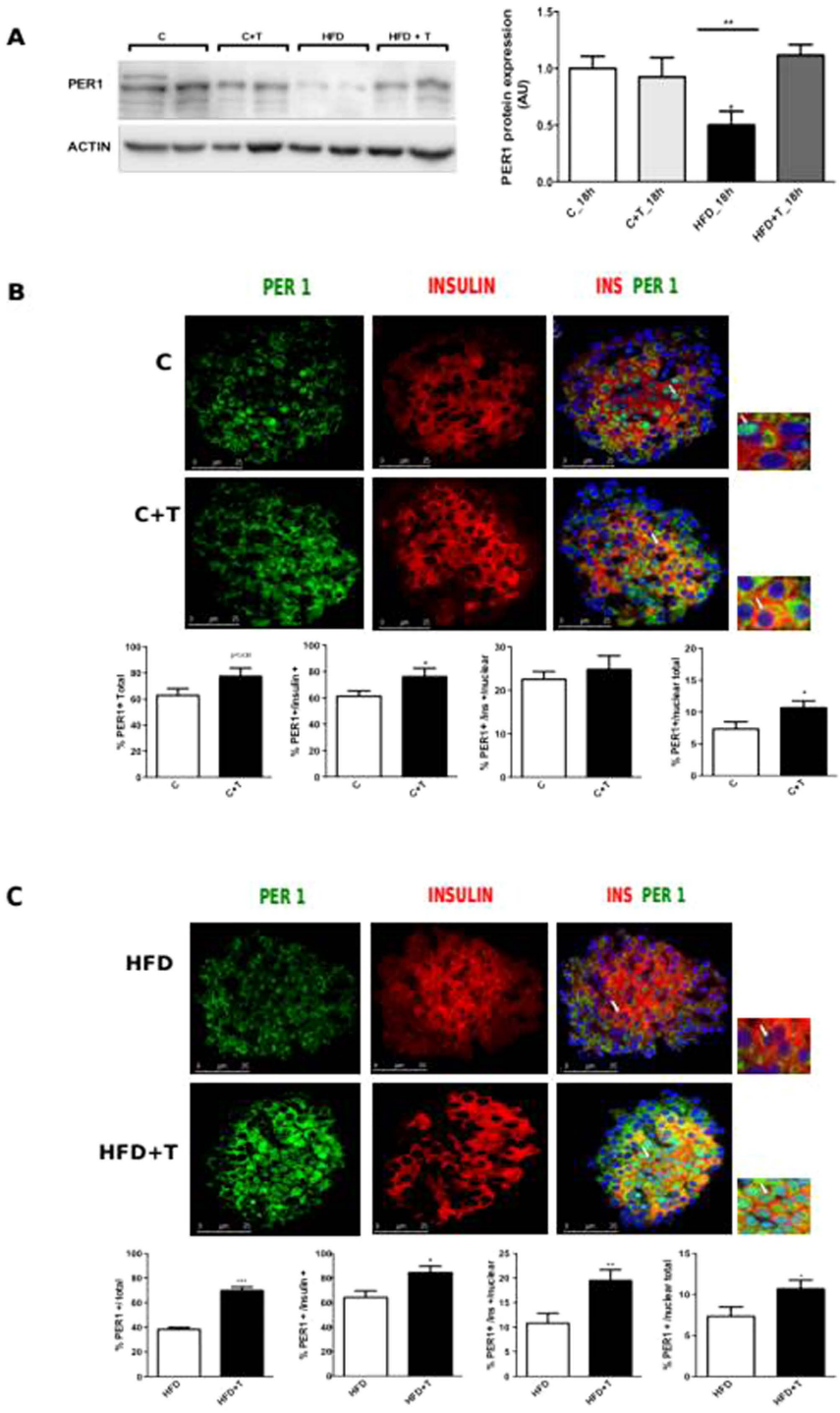

Figure 6. Effects of taurine in the expression of PER1 protein in isolated pancreatic islets. Pancreatic islets were isolated at 18:00 h after 10 weeks of taurine treatment in all experimental groups. Protein expression was detected by western blot and immunofluorescence stained against insulin anti-body (red), PER1 anti-body (green) and nuclear fraction by DAPI (blue). (**A**) PER1 protein expression normalized by actin. (n = 4–5 mice per group). (**B**) Percentage of PER1 expression in total number of cells, percentage of PER1 expression in the cytoplasm and the nucleus of insulin-positive cells, percentage of nuclear PER1 in beta-cells and percentage of nuclear PER1 in the total number of cells in chow diet groups. Differences between C (white bars) versus C+T (black bars). Data are expressed as mean ± SEM. Differences between C vs C+T (*$P < 0.05$) (n = 15–20 islets analyzed per condition per group). (**C**) Percentage of PER1 expression in total number of cells, percentage of PER1 expression in the cytoplasm and the nucleus of insulin-positive cells, percentage of nuclear PER1 in beta-cells and percentage of nuclear PER1 in the total number of cells in high fat diet groups. Differences between HFD (white bars) versus HFD+T (black bars). Data are expressed as mean ± SEM. Differences between HFD vs HFD+T (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 15–20 islets analyzed per condition per group).

Name	Sense primer (5′3)	Antisense primer (5′3)
Rev-erbα	GGTGCGCTTTGCATCTT	GGTTGTGCGGCTCAGGAA
Clock	TTGCTCCACGGGAATCCTT	GGAGGGAAAGTGCTCTGTTGTAG
Bmal1	GGACTTCGCCTCTACCTGTTCA	AACCATGTGCGAGTGCAGGCGC
Per1	GCGGGTCTTCGGTTAAGGTT	GCTCAGCTGGGATTTGG
Per2	ATGCTCGCCATCCACAAGA	GCGGAATCGAATGGGAGAAT
36b4	GAGGAATCAGATGAGGATATGGGA	AAGCAGGCTGACTTGGTTGC

Table 4. Quantitative real PCR primers.

with DAPI (Life Technologies, USA). Immunofluorescence was assessed in a confocal laser microscope (Leica. Microsystems, Wetzlar Germany). For each individual islet images were acquired every 10 μm using a 40x oil immersion objective.

The same settings (i.e pinhole, smart gain, smart offset, phase, zoom) were maintained for each islet in all groups. Images were analyzed by Image J Software 1.50a (NIH, USA). Analysis was done by counting the number of stained positive cells represented by percentage.

Statistical analysis. Values are presented as means ± SEM. Differences between two groups were analyzed by Student's *t* test. The effect of time and groups differences were measured by one-way or two-way ANOVA, with Bonferroni post hoc test for multiple comparisons, using GraphPad Prism software where appropriate. All statistical tests were performed with a level of significance $P < 0.05$. Cosinor Analysis was done using the Acrophase program (R. Refinetti 2004) for fitting cosine functions to the data using a fixed 24 hour period and included the mesor (middle value of the fitted cosine representing a rhythm-adjusted mean), the amplitude (difference between the minimum and maximum of the fitted cosine function), the acrophase (the time at which the peak of a rhythm occurs, expressed in hours) and fitted cosine values to calculate the goodness of the fit by coefficient of determination R^2.

References

1. Golombek, D. A. & Rosenstein, R. E. Physiology of circadian entrainment. *Physiological reviews* **90,** 1063–1102, doi: 10.1152/physrev.00009.2009 (2010).
2. Jones, B. Circadian genetics: Timing is everything. *Nat Rev Genet* **15,** 780–780, doi: 10.1038/nrg3864 (2014).
3. Lowrey, P. L. & Takahashi, J. S. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annual review of genomics and human genetics* **5,** 407–441, doi: 10.1146/annurev.genom.5.061903.175925 (2004).
4. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* **418,** 935–941, doi: 10.1038/nature00965 (2002).
5. Vieira, E., Burris, T. P. & Quesada, I. Clock genes, pancreatic function, and diabetes. *Trends in molecular medicine* **20,** 685–693, doi: 10.1016/j.molmed.2014.10.007 (2014).
6. Ando, H. *et al.* Impairment of peripheral circadian clocks precedes metabolic abnormalities in ob/ob mice. *Endocrinology* **152,** 1347–1354, doi: 10.1210/en.2010-1068 (2011).
7. Balsalobre, A., Marcacci, L. & Schibler, U. Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. *Current biology: CB* **10,** 1291–1294 (2000).
8. Marcheva, B. *et al.* Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* **466,** 627–631, doi: 10.1038/nature09253 (2010).
9. Muhlbauer, E., Wolgast, S., Finckh, U., Peschke, D. & Peschke, E. Indication of circadian oscillations in the rat pancreas. *FEBS letters* **564,** 91–96, doi: 10.1016/S0014-5793(04)00322-9 (2004).
10. Vieira, E. *et al.* The clock gene Rev-erbalpha regulates pancreatic beta-cell function: modulation by leptin and high-fat diet. *Endocrinology* **153,** 592–601, doi: 10.1210/en.2011-1595 (2012).
11. Vieira, E. *et al.* Involvement of the clock gene Rev-erb alpha in the regulation of glucagon secretion in pancreatic alpha-cells. *PloS one* **8,** e69939, doi: 10.1371/journal.pone.0069939 (2013).
12. Vieira, E. *et al.* Relationship between AMPK and the transcriptional balance of clock-related genes in skeletal muscle. *American journal of physiology. Endocrinology and metabolism* **295,** E1032–E1037, doi: 10.1152/ajpendo.90510.2008 (2008).
13. Perelis, M. *et al.* Pancreatic beta cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. *Science* **350,** aac4250, doi: 10.1126/science.aac4250 (2015).
14. Hirota, T. *et al.* Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. *The Journal of biological chemistry* **277,** 44244–44251, doi: 10.1074/jbc.M206233200 (2002).
15. Orozco-Solis, R. *et al.* Perinatal nutrient restriction induces long-lasting alterations in the circadian expression pattern of genes regulating food intake and energy metabolism. *International journal of obesity* **35,** 990–1000, doi: 10.1038/ijo.2010.223 (2011).
16. Rutter, J., Reick, M., Wu, L. C. & McKnight, S. L. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* **293,** 510–514, doi: 10.1126/science.1060698 (2001).
17. Bonfleur, M. L. *et al.* Improvement in the expression of hepatic genes involved in fatty acid metabolism in obese rats supplemented with taurine. *Life Sci.* **135,** 15–21 (2015).
18. Chen, W., Guo, J., Zhang, Y. & Zhang, J. The beneficial effects of taurine in preventing metabolic syndrome. *Food Funct*. doi:10.1039/c5fo01295c (2016).
19. Qian, J., Block, G. D., Colwell, C. S. & Matveyenko, A. V. Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. *Diabetes* **62,** 3469–3478, doi: 10.2337/db12-1543 (2013).
20. Podrini, C. *et al.* High-fat feeding rapidly induces obesity and lipid derangements in C57BL/6N mice. *Mammalian genome: official journal of the International Mammalian Genome Society* **24,** 240–251, doi: 10.1007/s00335-013-9456-0 (2013).
21. Wang, C. Y. & Liao, J. K. A mouse model of diet-induced obesity and insulin resistance. *Methods in molecular biology* **821,** 421–433, doi: 10.1007/978-1-61779-430-8_27 (2012).
22. Batista, T. M. *et al.* Taurine supplementation improves liver glucose control in normal protein and malnourished mice fed a high-fat diet. *Molecular nutrition & food research* **57,** 423–434, doi: 10.1002/mnfr.201200345 (2013).
23. Branco, R. C. *et al.* Long-term taurine supplementation leads to enhanced hepatic steatosis, renal dysfunction and hyperglycemia in mice fed on a high-fat diet. *Advances in experimental medicine and biology* **803,** 339–351, doi: 10.1007/978-3-319-15126-7_26 (2015).

24. Nakaya, Y. *et al.* Taurine improves insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous type 2 diabetes. *The American journal of clinical nutrition* **71,** 54–58 (2000).
25. Nardelli, T. R. *et al.* Taurine prevents fat deposition and ameliorates plasma lipid profile in monosodium glutamate-obese rats. *Amino acids* **41,** 901–908, doi: 10.1007/s00726-010-0789-7 (2011).
26. Tsuboyama-Kasaoka, N. *et al.* Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. *Endocrinology* **147,** 3276–3284, doi: 10.1210/en.2005-1007 (2006).
27. Cano, P. *et al.* Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. *Endocrine* **33,** 118–125, doi: 10.1007/s12020-008-9066-x (2008).
28. Kohsaka, A. *et al.* High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell metabolism* **6,** 414–421, doi: 10.1016/j.cmet.2007.09.006 (2007).
29. Camargo, R. L. *et al.* Effects of taurine supplementation upon food intake and central insulin signaling in malnourished mice fed on a high-fat diet. *Advances in experimental medicine and biology* **776,** 93–103, doi: 10.1007/978-1-4614-6093-0_10 (2013).
30. Solon, C. S. *et al.* Taurine enhances the anorexigenic effects of insulin in the hypothalamus of rats. *Amino acids* **42,** 2403–2410, doi: 10.1007/s00726-011-1045-5 (2012).
31. Boden, G., Chen, X. & Polansky, M. Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. *Diabetes* **48,** 2182–2188 (1999).
32. Boden, G., Ruiz, J., Urbain, J. L. & Chen, X. Evidence for a circadian rhythm of insulin secretion. *The American journal of physiology* **271,** E246–E252 (1996).
33. Costa Justus, J. F. *et al.* Early Effect of Bariatric Surgery on the Circadian Rhythms of Adipokines in Morbidly Obese Women. *Metabolic syndrome and related disorders* **14,** 16–22, doi: 10.1089/met.2015.0051 (2016).
34. Kalsbeek, A. & Strubbe, J. H. Circadian control of insulin secretion is independent of the temporal distribution of feeding. *Physiology & behavior* **63,** 553–558 (1998).
35. Kalsbeek, A., Yi, C. X., La Fleur, S. E. & Fliers, E. The hypothalamic clock and its control of glucose homeostasis. *Trends in endocrinology and metabolism: TEM* **21,** 402–410, doi: 10.1016/j.tem.2010.02.005 (2010).
36. La Fleur, S. E., Kalsbeek, A., Wortel, J. & Buijs, R. M. A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *Journal of neuroendocrinology* **11,** 643–652 (1999).
37. Yildiz, B. O., Suchard, M. A., Wong, M. L., McCann, S. M. & Licinio, J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proceedings of the National Academy of Sciences of the United States of America* **101,** 10434–10439, doi: 10.1073/pnas.0403465101 (2004).
38. Ahren, B. Diurnal variation in circulating leptin is dependent on gender, food intake and circulating insulin in mice. *Acta physiologica Scandinavica* **169,** 325–331, doi: 10.1046/j.1365-201x.2000.00746.x (2000).
39. Allaman-Pillet, N. *et al.* Circadian regulation of islet genes involved in insulin production and secretion. *Molecular and cellular endocrinology* **226,** 59–66, doi: 10.1016/j.mce.2004.06.001 (2004).
40. Pulimeno, P. *et al.* Autonomous and self-sustained circadian oscillators displayed in human islet cells. *Diabetologia* **56,** 497–507, doi: 10.1007/s00125-012-2779-7 (2013).
41. Ribeiro, R. A. *et al.* Taurine supplementation prevents morpho-physiological alterations in high-fat diet mice pancreatic beta-cells. *Amino acids* **43,** 1791–1801, doi: 10.1007/s00726-012-1263-5 (2012).
42. Santos-Silva, J. C. *et al.* Taurine supplementation ameliorates glucose homeostasis, prevents insulin and glucagon hypersecretion, and controls beta, alpha, and delta-cell masses in genetic obese mice. *Amino acids* **47,** 1533–1548, doi: 10.1007/s00726-015-1988-z (2015).
43. Lee, J. *et al.* Bmal1 and beta-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced beta-cell failure in mice. *Molecular and cellular biology* **33,** 2327–2338, doi: 10.1128/MCB.01421-12 (2013).
44. Rakshit, K., Hsu, T. W. & Matveyenko, A. V. Bmal1 is required for beta cell compensatory expansion, survival and metabolic adaptation to diet-induced obesity in mice. *Diabetologia*, doi: 10.1007/s00125-015-3859-2 (2016).
45. Yanagihara, H., Ando, H., Hayashi, Y., Obi, Y. & Fujimura, A. High-fat feeding exerts minimal effects on rhythmic mRNA expression of clock genes in mouse peripheral tissues. *Chronobiol. Int.* **23,** 905–914 (2006).

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Author Contributions

Designed the study: E.V., R.G. Performed the experiments: A.F., H.F., S.R. Analyzed the data: A.F., H.F., E.V. Interpreted results: A.F., H.F., E.V., R.G. Supervised the project: E.V. and R.G. Wrote the paper: A.F., E.V. Revised and edited the manuscript. E.V., R.G. Provided reagents and financial support R.G. All authors approved the final manuscript.

Additional Information

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