Brain, nutrition and metabolism

Studies in lean, obese and insulin resistant humans

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Serotonin, a possible intermediate between disturbed circadian rhythms and metabolic disease

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Abstract

It is evident that eating in misalignment with the biological clock (such as in shift work, eating late at night and skipping breakfast) is associated with increased risk for obesity and diabetes. The biological clock located in the suprachiasmatic nucleus dictates energy balance including feeding behavior and glucose metabolism. Besides eating and sleeping patterns, glucose metabolism also exhibits clear diurnal variations with higher blood glucose concentrations, glucose tolerance and insulin sensitivity prior to waking up. The daily variation in plasma glucose concentrations in rats, is independent of the rhythm in feeding behavior. On the other hand, feeding itself has profound effects on glucose metabolism, but differential effects occur depending on the time of day. We here review data showing that a disturbed diurnal eating pattern results in alterations in glucose metabolism induced by a disrupted circadian clock. We first describe the role of central serotonin on feeding behavior and glucose metabolism and subsequently describe the effects of central serotonin on the circadian system. We next explore the interaction between the serotonergic system and the circadian clock in conditions of disrupted diurnal rhythms in feeding and how this might be involved in the metabolic dysregulation that occurs with chronodisruption.
Introduction

The prevalence of obesity has increased progressively over the past decades and this has been linked to several serious medical conditions including Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease (1). In essence obesity is caused by a mismatch between energy intake and expenditure. The brain, especially the hypothalamus, plays a central role in the homeostatic control of energy balance. The hypothalamus not only integrates peripheral signals on energy status, but also orchestrates feeding and autonomic responses to maintain body weight and blood glucose at the appropriate level. To do so different hypothalamic nuclei integrate information from nutrients and related hormonal signals via many neuronal pathways using a variety of (peptidergic) neurotransmitters (2, 3).

The basal output of the hypothalamus is not constant but shows diurnal variation. The rhythmicity in output is orchestrated by the biological clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus. In this respect, besides eating and sleeping patterns, glucose metabolism also exhibits clear diurnal variations with higher blood glucose concentrations, glucose tolerance and insulin sensitivity prior to waking up (4-6) and feeding-induced responses in glucose metabolism depending on the time of day (7). Although feeding behavior has profound effects on glucose metabolism the diurnal variation in blood glucose concentrations in rats, is independent of the rhythm in feeding behavior (8, 9). Generated by pacemaker cells, the SCN has an intrinsic rhythm of a little more than 24h (in rats and humans), which is synchronized to the exact 24-h rhythm of the environment by the effect of light on specialized photosensitive ganglion cells in the retina expressing the photopigment melanopsin. These ganglion cells directly innervate the SCN via the retinohypothalamic tract (RHT) and indirectly via the geniculohypothalamic tract and the intergeniculate leaflet (IGL) (10). In addition to photic input, the SCN also receives non-photic input on arousal or activity state from the serotonergic cells located in the raphe nuclei either via a direct raphe - SCN projection or indirectly via the IGL (11-14). The SCN projects to a wide range of nuclei, mostly within the hypothalamus, which allows for transferring rhythmic signals to both the autonomic nervous system and the hormonal output (Fig 1)(15). In addition to the central clock in the SCN, circadian oscillators are localized in (almost) all cells of the body, including in organs involved in glucose metabolism, such as the liver, adipose tissue, pancreas and skeletal muscle. The peripheral clocks receive synchronizing signals from the central clock in the SCN, but their main synchronizer seems to be the fasting-feeding cycle (16) which is sufficient to drive the circadian expression of these peripheral oscillators (17). Thus, diurnal regulation of energy balance and metabolism is dictated by a complex of circadian players, including the SCN, peripheral clocks, and rhythms in light exposure and feeding behavior. In this network light influences the SCN to entrain the organism to the environmental light/dark cycle, and the SCN in its turn enables the body to anticipate upcoming
events such as awakening in the morning. In addition, feeding itself evokes metabolic and endocrine responses that affect central and peripheral circadian clocks that can respond with either phase-delays or phase-advances according to their phase during exposure (18). When feeding is in synchrony with the environmental light/dark cycle, the SCN and the peripheral clocks will be aligned and the body’s physiology will be able to anticipate and exert its metabolic and hormonal responses appropriately to the time of the day.

In contrast, feeding in misalignment with the biological clock such as feeding in the dark for humans or during light conditions in rodents will result in conflicting signals to the SCN, because the light conditions are in conflict with the metabolic state. In addition to this discrepancy in input between the central and peripheral oscillators, eating at an inappropriate time point of the day can also cause desynchrony at the level of the SCN because besides photic feedback the SCN also receives non-photic feedback, including non-photic neural feedback derived from serotonin producing cells in the raphe nuclei of the midbrain. The raphe nucleus and serotonin levels themselves are sensitive to nutrients, providing information on which macronutrients are consumed at what time of the day. Thus in diurnal species feeding at night will signal daytime in the SCN via the raphe – SCN projections at times that the light/dark cycle signals darkness via the retinohypothalamic tract (RHT) (19-22).

We here propose that the observed increased risk for metabolic disturbances in subjects consuming food at inappropriate times of the day (from a SCN point of view) is explained by a mismatch in input from the raphe-SCN and RHT-SCN with a major role for serotonin. We will first review the role of serotonin in feeding behavior and glucose metabolism and subsequently describe the effects of serotonin on the circadian system. We next explore the interaction between these two systems in conditions of misalignment such as eating during night time for humans or during daytime for rodents. We further discuss how this might be involved in metabolic dysregulation.

**Serotonin’s role in feeding behavior and glucose metabolism**

In the brain, serotonin (5-hydroxytryptamine (5-HT)) is produced in the dorsal and medial raphe nucleus (DRN and MRN, respectively) with widespread projections throughout the brain including several nuclei within the hypothalamus (23-25). Serotonin is synthesized from the essential amino acid tryptophan which is hydroxylated by tryptophan hydroxylase to 5-hydroxytryptophan (5-HTP), which in its turn is decarboxylated to form serotonin (5-HT). After storage in vesicles and release in the synaptic cleft via exocytosis, 5-HT is metabolized to 5-hydroxyindoleacetic (5-HIAA) by monoamine oxidase (MAO) and aldehyde dehydrogenase (26, 27). Serotonin signaling is regulated by 17 serotonin receptors, which are almost all G-protein coupled, and a serotonin transporter which is located on the presynaptic neuron and reduces serotonin availability by facilitating the reuptake into the presynaptic neuron (28).
Although involved in many physiological processes, one of the main effects of central serotonin is to inhibit feeding behavior (29, 30). In rodents, for example, central administration of 5-HT decreases food intake (31, 32), whereas selective depletion of central 5-HT is associated with hyperphagia and increased body weight (33, 34). Also in humans, serotonin depletion has been associated with increased feeding behavior (35), although 5HIAA, a 5-HT metabolite, was found to be significantly elevated in cerebrospinal fluid (CSF) of overweight subjects (36). The receptors involved in serotonin’s inhibitory effects on feeding are mostly the 5-HT\textsubscript{1A}R and 5-HT\textsubscript{2C}R subtypes. 5-HT\textsubscript{2C}R-/- mice show hyperphagia, increased body weight and an increased risk for developing insulin resistance (37, 38) and 5-HT\textsubscript{1B}R and 5-HT\textsubscript{2A}R mutations result in hyperphagia with a mild increase in body weight (39, 40). Interestingly, 5-HT\textsubscript{1B}R knockout mice show reduced 5-HT\textsubscript{2C}R sensitivity, as these mice are resistant to hypophagia induced by 5-HT\textsubscript{2C}R agonists (41). In addition, 5-HT\textsubscript{6}R knockout mice show reduced feeding behavior and body weight gain (42) and others have shown that they are resistant to weight gain when exposed to a high-fat diet (43). These three receptors are the most effective when targeted pharmacologically to alter feeding behavior in rodents and have been of interest for human clinical trials very recently. For example, both 5-HT\textsubscript{2C}R and the 5-HT\textsubscript{1A}R antagonists block the inhibitory effects of the nonspecific agonist flenfuramine on food intake (44-50). Furthermore, 5-HT\textsubscript{2C}R, 5HT\textsubscript{1B}R and 5-HT\textsubscript{1C}R specific agonists all reduce food intake (51-54), while the 5-HT\textsubscript{6}R receptor antagonists suppress food intake in rats (55). As the 5-HT\textsubscript{6}R receptor is exclusively expressed in the central nervous system (CNS) (56), drugs targeted at these receptors may have relatively few side-effects in the periphery. Finally, the 5-HT\textsubscript{1A}R agonist increases feeding which is most likely the result of inhibitory autoreceptor activity on raphe neurons (57). For human research trials, the 5-HT\textsubscript{2C}R and 5-HT\textsubscript{6}R have been given the most attention; Lorcaserin, a centrally acting, selective serotonin 5-HT\textsubscript{2C} receptor full agonist, reduced weight during a 12 week phase IIb study (58). Placebo-controlled trials, each lasting one year, in a total of about 6000 patients, confirmed these effects (59, 60). Lorcaserin appears to be well tolerated in patients and the most common adverse events reported did not include serious complications.

In addition to serotonin receptors, serotonin transporters (SERT) have also been shown to be involved in feeding behavior and body weight regulation. Deficiency of SERT in mice leads to late-onset obesity with development of the metabolic syndrome (61) and in female rats it was shown that SERT deficiency resulted in increases in body adiposity (62). Moreover, serotonin reuptake inhibitors (SSRI), such as sertraline and fluoxetine, which increase extra cellular serotonin availability and thus enhance 5-HT signaling, decrease food intake in rats and result in body weight loss (63, 64). In humans, sibutramine reduces body weight in 77% of obese people after 2 years (65-68). Taken together, both animal and human studies provide a wide support for a role of three specific 5-HT receptors and the serotonin transporters in serotonin’s inhibitory effects on feeding behavior. It has to be noted, however, that although SSRIs acutely reduce food intake, long-term use is associated with weight gain (69, 70). This longterm effect that is insufficiently studied and underlying mechanisms remain unclear.
As the hypothalamic nuclei involved in regulating feeding behavior are also involved in regulating glucose metabolism, it is not surprising that recent attention within serotonin research also has taken into account its involvement in regulating glucose metabolism. Although it has already been known since the 70s that injections of L-tryptophan or 5-HT in rodents lead to hypoglycemia (71-73), and injection of a 5-HT antagonist results in hyperglycemia (74), a clear role for the hypothalamus in these effects was demonstrated more recently. Mice lacking the 5-HT$_{2C}$R in pro-opiomelanocortin (POMC) neurons, located in the arcuate nucleus of the hypothalamus (ARC), have normal body weight but show hyperglycemia, hyperinsulinemia and insulin resistance pointing to a clear role for central serotonin signaling in regulating glucose metabolism (75). Besides, SERT deficient mice are hyperglycemic, hyperinsulinemic and have reduced insulin signaling in the liver prior to the onset of obesity (61). Also in humans, serotonin has been implicated to be involved in glucose regulation as in patients with non-insulin-dependent T2DM pharmacological manipulation of 5-HT levels with the drugs fenfluramine and dexfenfluramine increased insulin sensitivity, reduced hepatic glucose production and decreased visceral fat mass (76-80), although an independent effect on glucose metabolism remains to be demonstrated. Also short term treatment with SSRIs have been shown to affect glucose metabolism with anti-hyperglycemic effects in rats (81) and an improvement of insulin sensitivity in human with non-insulin-dependent T2DM, independent of weight loss (82-84). However, some studies showed that long-term SSRI use induces insulin resistance in rodents (61, 85) and leads to an increased risk of T2DM in humans (86, 87), which could be due to the increased body weight gain that also has been reported.

Many studies on serotonin's involvement in feeding regulation and glucose metabolism involve whole body manipulations of serotonin and thus it is difficult to point to a specific role for brain serotonin in feeding behavior, since 90% of bodily 5-HT is expressed in the intestine (88). However, very recently it was shown that inhibiting serotonin synthesis in the intestines (leaving serotonin production in the brain intact) resulted in animals that were protected from obesity and metabolic disturbances (89), pointing towards a differential role for peripheral and central serotonin in body weight regulation as a reduction of serotonin synthesis in the brain increases feeding behavior(29).

**Effects of feeding behavior and macronutrients on the serotonin system**

Not only has serotonin profound effects on feeding behavior and metabolism, feeding behavior itself as well as nutritional status, obesity and ingestion of macronutrients all have clear effects on the serotonin system. In rodents, a high palatable diet increases the density of 5-HT$_{1A}$ presynaptic receptors in the raphe, suggesting a decrease in synthesis and release of 5-HT, which might ultimately result in less inhibition of feeding (90). Within the hypothalamus it has been shown that fat and protein intake reduce, while carbohydrates increase, 5-HT levels (91, 92). Moreover, increases in the number of 5-HT$_{1B}$ presynaptic receptors in the ARC of diet-induced obese rodents have also been reported, further pointing to an increased inhibition of the system through these autoreceptors (90, 93). Taken
together these data point to reduced hypothalamic serotonergic signaling when consuming high fat/palatable food, further stimulating overeating and obesity. Although in humans effects on the serotonergic system in the brain are more difficult to assess, imaging the binding of radioactive ligands to 5-HT receptors and SERT in the brain using SPECT and PET-CT has shown a positive correlation between BMI and 5-HT$_{2A}$R binding in the temporal and frontal cortex in normal weight people (94). Moreover a positive correlation between BMI and 5-HT$_{2A}$R binding in the cerebral cortex in obese people has been found (95), as well as a positive correlation between BMI and 5-HT$_{4}$R density bilaterally in the nucleus accumbens and ventral pallidum, and additionally in the left hippocampal region and orbitofrontal cortex (96). Night eaters show significantly greater SERT binding in the midbrain than healthy controls (97) and SERT binding is decreased in the mid-brain of binge-eating women compared to obese controls (98). Erritzoe showed a negative correlation between cortical and subcortical SERT binding and BMI (99), however, in high BMI twins hypothalamic SERT binding was higher than in leaner control twins (100). It therefore seems that changes in components of the serotonin system in obesity or eating disorders are region specific and it is difficult to assess whether changes are a consequence of the eating disorder/obesity or whether alterations in the serotonin system underlie the disorder. Moreover, in line with the palatable feeding studies in rodents that point to reduced serotonin signaling when overeating, we recently showed that a hypercaloric high-fat-high-sugar (HFHS) diet of 6 weeks leads to a 30% decrease of SERT binding in the dienencephalon when the excess calories where consumed in between the meals and in the evening (snacking diet)(101). This effect was not present in the males on an isocaloric high sugar snacking diet, suggesting that indeed macronutrients and timing of feeding have independent effects on the brain serotonergic system in humans.

In short, brain serotonin signaling is linked to food intake, body weight regulation and glucose metabolism. Alternatively, specific macronutrients and eating patterns modulate serotonergic signaling suggesting a mutual interaction. Another interaction is clear for the circadian and serotonergic system in relation to feeding behavior (102).

**Serotonergic-circadian interactions**

Levels of serotonin in several brain regions show circadian rhythmicity, including the SCN, pineal gland, raphe nuclei and striatum. The secretion of 5-HT in the SCN by the raphe terminals as well as the diurnal 5-HT rhythms in MRN and DRN change over the light/dark schedule characterized by a sharp increase in release during late midday to peak levels at the light/dark transition in hamsters and rats (103-105). Furthermore, in vivo peak levels of serotonin in plasma and the hypothalamus during the dark phase (106) and diurnal variations of serotonin receptor subtypes in the rat hippocampus and cortex were observed and using in vivo voltammetry it was shown that also serotonin release was rhythmic in these brain sites (107). Systemic administration of a 5-HT$_{1A}$ agonist showed a significantly greater suppressive effect on 5-HT release in the hamster SCN using in vivo microdialysis during the late dark phase compared with the mid light phase. This might explain the variation in raphe autoreceptor response and may
underlie the time-dependent effects of wheel running on 5-HT release (104). Also SERT mRNA expression and in vivo 5-HT uptake activity in the mouse midbrain showed significant time-dependent changes with higher levels during the dark phase and lower levels during the light phase in raphe nuclei (108, 109). These results suggest that the re-uptake of 5-HT might be increased during the dark phase. In the raphe nuclei, in vivo diurnal rhythms in tryptophan hydroxylation have been found and in situ studies showed rhythms in the catalyzing enzymes tryptophan hydroxylase 1 (TPH1) and TPH2 with a peak during the dark period, suggesting rhythmic control of 5-HT biosynthesis (110-112). The main metabolite of serotonin, 5-HIAA, also shows diurnal variation in the hamster SCN as measured by in vivo microdialysis, with increased release of 5-HIAA during the dark as compared to the light phase (113). Of note, most of the studies outlined above did not account for effects of feeding behavior which could have affected outcome as tryptophan is consumed via the diet.

The 5-HT cell bodies in the raphe provide direct and indirect (via the IGL of the thalamus) non-photic projections to the SCN (114-116). Electrical stimulation of the DRN or MRN in hamsters evoked 5-HT release in the SCN, which is blocked by systemic injection of 5-HT antagonists (117). Key molecules in the serotonin signaling network, such as SERT (118, 119) and different serotonin receptors (5-HT1B, 5-HT2C and 5-HT7 receptors) are expressed in the SCN where they may mediate serotonin’s effects on rhythms (120, 121). Serotonergic agonists and serotonin reuptake inhibitors (e.g., fluoxetine) have a non-photic influence (phase-shifting effects during daytime and attenuation of photic resetting during nighttime) on the hamster and mice central clock. Activation of both 5-HT1A, 5-HT5A and 5-HT7 receptors can produce phase advances in locomotor activity rhythms in hamsters (122-125) and induce phase advances of SCN neuronal firing in vitro (Sprouse et al., 2004). Furthermore, 5-HT agonists administered directly into the SCN (both in vivo and in vitro) modulate behavioral circadian phase resetting (126). These effects are time dependent: in vitro serotonin activation induces a 2-3 h phase advances when applied during subjective day (i.e. the ‘daylight’ segment under free running conditions), while non-significant phase shifts were seen after 5-HT administration at other times of day and these phase shifts were completely blocked by the 5-HT antagonist metergoline, thus demonstrating that the mouse SCN, like that of the rat, is directly sensitive to phase-resetting by 5-HT (127). However, this effect is species specific as another study did not find a phase shift in locomotor activity after injections of the 5-HT1A antagonist in the hamster, despite significantly increased 5-HT levels in the SCN (128).

The 5-HT release in the SCN is behaviorally regulated. Serotonin receptor activity in the raphe can be defined as an arousal-dependent factor because the (single unit) activity of the serotonergic neurons and in vivo serotonin release are closely correlated with the level of behavioral arousal and locomotor activity (129). Manipulations that reset the behavioral phase evoke SCN 5-HT release mediated by 5-HT7 and GABAergic receptors of the DRN and MRN (126). Furthermore, 5-HT depletion in the MRN (but not in the DRN) induces an advance in onset, a delay in
offset, and a longer duration of the nocturnal running-wheel activity phase (114). Paulus and Mintz showed that loss of serotonergic input to the SCN in mice has long-term consequences for both circadian clock parameters and the temporal organization of activity (130), however, depletion of 5-HT in the SCN itself does not block behavioral phase shifting, which supports the existence of indirect effects of 5-HT on the SCN and/or the existence of alternative mechanisms. In summery serotonin agonists and antagonists can modulate many aspects of circadian rhythmicity in animal models, supporting the involvement of serotonin turnover in circadian rhythm regulation.

**Chronodisruption and metabolic disturbances**

Humans eat during the day, and a three-meal-a-day pattern is universal and can be traced back for centuries (131). Rats and mice with ad libitum access to food consume most of the food during the night (dark period). Such feeding patterns are under SCN influence as bilateral SCN lesions or knife cuts around the SCN in rats abolish the daily rhythmicity of feeding (132-134). In addition, food intake is also affected by light: in rodents, light exposure at night, the active period, acutely reduces food intake and dark exposure during the day time, the inactive phase, enhances food intake. However, it is not the light/dark-cycle per se that is causing the feeding rhythm; when rats are exposed to a short light pulse every 12h (to signal at dawn and dusk) but remain in darkness, they maintain their diurnal feeding rhythm with consuming food in the subjective night. Moreover, in constant darkness a clear free running circadian rhythm is apparent with a period of a little over 24h (134).

In turn food availability can impact rhythmicity. When food is limited to a couple of hours every day at the same time, animals will anticipate with higher locomotor activity, body temperature and corticosterone secretion prior to food availability, suggesting a time cue for behavioral and physiological processes. Interestingly, this anticipation persists in SCN lesioned animals and on restricted feeding schedules. These food-sensitive components of the circadian timing system are referred to as food-entrainable oscillators. Despite enormous efforts of many groups, the essential brain areas involved in food entrainment remain to be identified (135). In addition also peripheral oscillators, like in the liver, are sensitive to feeding and synchronize to a feeding schedule. For example, when food is limited to the light period, the inactive phase for mice, clock protein and mRNA rhythms in the liver shift to the time of feeding, however, the clock gene rhythms in the SCN will not shift, resulting in a misalignment of liver and SCN clock rhythms (16). Interestingly, shifting the availability of food to the inactive period in mice results in an obese phenotype; mice fed a high-fat diet during the 12-h light phase gain significantly more weight than mice fed during the 12-h dark phase not explained by changes in activity or caloric intake (136). Furthermore, two weeks of eating in the light period leads to perturbations in glucose and lipid metabolism in a rodent model of shiftwork but subsequently restricting food intake to the dark phase, while still on the shiftwork schedule (i.e. working in the light), reduced body weight and reverses metabolic and rhythmic disturbances.
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Moreover, early nocturnal meal skipping alters peripheral liver clocks and increases lipogenesis in mice (139) and mice that consumed one large meal at the beginning of the awake period (breakfast only) exhibited an increase in body weight gain, hyperinsulinemia, hyperleptinemia, and a decrease of gene expression involved in β-oxidation in adipose tissue and liver compared with those on two meals (a bigger breakfast at the beginning of the dark (active) phase with a smaller dinner at the end of the dark phase)(140). Taken together, the rodent data show that feeding in the light (inactive) period and frequency of meals during the dark (active) period plays a role in body weight gain and metabolic disturbances. It remains to be elucidated what the roles of peripheral versus central clock rhythms are regarding this phenomena.

Studies performed in humans have shown similar results and postulated that consuming food at inappropriate times of the day contributes to the development of obesity for (for review: (18)). Many epidemiological studies show that circadian disruption induced by shift work or shifting the normal feeding time to night hours, is associated with an increased risk of obesity, type 2 diabetes and cardiovascular diseases (141, 142). Although night workers show a partial adaptation of their 24-h glucose and insulin sensitivity rhythm (143) they have abnormal metabolic responses to meals taken at night with higher plasma glucose and triglyceride levels in part explained by lower insulin sensitivity (144). Besides shift workers, modern society shifts towards a more active nocturnal life style with food intake (and snacking) later during the evening and night, thus at times when circadian systems should initiate a period of fasting and rest. Moreover, in recent years, humans more frequently skip breakfast, perhaps in part due to a belief that skipping breakfast can help reducing total energy intake and controlling body weight. However, many studies showed that on the contrary regular breakfast consumption reduces total daily energy intake and is thereby associated with significant lower BMI. Omitting breakfast probably leads to weight gain because of a higher energy intake during the remainder of the day (145, 146). Moreover, skipping breakfast impairs fasting lipids and postprandial insulin sensitivity (147). Macronutrient composition of breakfast plays an important role as well: high carbohydrate breakfasts result in more weight loss associated with reduced hunger and craving scores compared to low carbohydrate breakfasts after a 32 weeks diet intervention study in obese people (148), and breakfasts high in whole grain and fiber content (e.a. ready-to-eat cereal) have a positive influence on appetite control, insulin resistance and mood (149). It was shown that eating more of the day's total energy intake at midday is associated with a lower risk of being overweight/obese, whereas consuming more in the evening (>33% of daily energy intake) is associated with a higher risk (150). In support of a role of timing of food intake in body weight regulation, a hypocaloric diet over 20 weeks which contained lunch before 3pm versus after 3pm resulted in significant more weight loss in the earlier lunch group (151). Consuming more calories at breakfast compared with at dinner had a more profound effect on body weight loss and improvement of insulin sensitivity and was associated with reduced hunger and craving scores as well as ghrelin levels (152). In contrast,
daily energy intake through snacking (eating episode not triggered by hunger but instead triggered by anything else) has increased over the last decades (153) and it has been shown that obese subjects more frequently display snacking habits compared to lean people (154). According to Palmer et al. there is no evidence for a role of eating frequency per se in weight-loss or maintenance interventions (155), but eating more often than three times a day was positively associated with total energy intake and being overweight, because the energy content of snacks was never compensated for by reduced food intake during the next meal (156, 157). Munsters and colleagues compared isocaloric low meal frequency (3 meals/day) regimen with that of an isocaloric high meal frequency (14 meal/day) regimen for 3 days and showed lower glucose excursions and increased resting metabolic rate and appetite control in the low meal frequency group (158). Farshchi and colleagues compared regular with irregular meal frequency diet in a cross-over study and found that peak insulin concentrations and AUC of insulin responses were significant higher after irregular meal patterns for 14 days (159, 160) and snacking in the evening was associated with a greater overall caloric intake and higher BMI, independently of chronotype of the subjects (161, 162). A cross-over study showed that a nocturnal life style for 3 weeks (shifting sleeping time, skipping breakfast and >50% of daily food intake in the evening), caused a prolonged increase of glucose concentrations and a decreased insulin secretion between midnight and morning (163).

Taken together, data from breakfast skippers, shift workers and animals point to an important role for eating in alignment with the circadian cycle to prevent disturbed glucose and lipid homeostasis and body weight gain. Indeed, Scheer et al showed that misalignment between behavioral (feeding/fasting and sleep/wake cycles) and endogenous circadian cycles in healthy volunteers resulted in metabolic complications such as altered postprandial glucose responses (164).

**Figure 1.** The SCN projects to a wide range of brain areas to influence both the autonomic nervous system and hormonal output in control of metabolism. SCN=suprachiasmatic nucleus; ARC=arcuate nucleus; MPO= medial preoptic area; PVN=paraventricular nucleus; DMH= dorsomedial hypothalamic nucleus; LHA=lateral hypothalamic area; PIT=pituitary; DMV=dorsomotor nucleus of the vagus; NTS= nucleus tractus solitarius; IML= intermedial lateral column.
Serotonin in the midst of circadian misalignment and metabolic disorders

As outlined above, eating in discordance with the biological clock and the light/dark cycle puts humans at risk for metabolic disease. Although a misalignment between the central clock and peripheral clocks such as in the liver might contribute to these effects, the SCN itself might play a major role in these metabolic consequences since the SCN receives both photic and non-photic information and thus light input will be differently phased than information on what and when we eat.

The SCN dictates glucose levels and insulin sensitivity to rise prior to awakening to ensure that we are prepared for the activity phase, and thus aligns glucose metabolism with the sleep/wake cycle. In addition to this anticipation in glucose metabolism to meet expected glucose demands, the SCN also influences, depending on the time of the day, the responses of pancreas and liver to abrupt glucose changes in rats (such as a glucose rise after a meal or hypoglycaemia) (7, 165). The SCN regulates diurnal rhythms in glucose metabolism via its projections to the autonomic nervous system (ANS) that innervates peripheral tissues (166, 167). And the SCN acts via both ANS and the anterior pituitary to set the endocrine system involved in the response to acute glucose challenges appropriate for the time of the day the challenge occurs (168). Within the hypothalamus several nuclei receiving SCN input have been shown to be involved in regulating glucose metabolism (169). Besides the paraventricular nucleus and the lateral hypothalamus receiving neural input from the SCN, also the ARC receives direct neural input (170). The ARC has been studied intensively in relation to its control over feeding behavior and glucose metabolism (171) as it is close to the blood brain barrier and is sensitive to hormones and nutrients which signal on energy status to maintain homeostasis. The ARC consists of two groups of neurons expressing either neuropeptide Y (NPY) and agouti related protein (AgRP), or POMC and cocaine amphetamine regulated transcript (CART). Like the SCN, also ARC neurons receive serotonergic input from the raphe and over 90% of ARC neurons are sensitive to serotonin (172). Both the 5-HT\textsubscript{1B}R and the 5-HT\textsubscript{2C}Rs are expressed in the ARC and both play a role in energy balance where the first one is expressed on NPY/AgRP neurons and the latter one on POMC neurons. Activation of the 5HT\textsubscript{1B}R hyperpolarizes neurons and inhibits neuropeptide release, whereas activation of 5-HT\textsubscript{2C}R depolarizes POMC neurons resulting in the release of alpha MSH (173). Moreover, in mice, 5-HT\textsubscript{2C}R deficiency abolished the anti-diabetic effects of meta-chlorofenylpiperazine (mCPP) and these effects were restored when 5-HT\textsubscript{2C}Rs were re-expressed in POMC neurons (75), again pointing to an important role for serotonin in the ARC in the regulation of glucose metabolism.

As outlined above, nutrient intake and its associated metabolic responses give feedback to the brain in order to maintain energy balance and to orchestrate the appropriate metabolic and feeding responses in relation to the time of the day. As the SCN controls rhythmicity of glucose metabolism which is important to align glucose metabolism to the active phase, metabolic feedback to the SCN is essential. Recently, the ARC has been put forward as a candidate to provide
metabolic feedback to the SCN as in addition to receiving input, it also sends neural projections to the SCN (174). Furthermore, activation of ARC neurons by administration of ghrelin (a peptide released by the stomach signaling hunger) provided support for ARC neurons transmitting feeding related signals to the SCN (170). Moreover, Saderi et al showed that NPY projections from the IGL to the SCN also provides feedback on metabolic conditions such as fasting and refeeding (175). We here would like to propose another possibility of metabolic feedback to the SCN concerning information about nutrients consumed and time of the day. This implies a major role of the serotonergic input to the SCN. The serotonin system directly innervates the SCN and is responding to arousal and (locomotor) activity, but also to nutrient intake, thus providing a setting in which information about feeding activity and macronutrients consumed are integrated and relayed to the SCN. Since, 5-HT$_{2c}$ receptors also reside within the SCN, the metabolic feedback from the raphe nuclei could result in modulatory control of glucose metabolism through the SCN. In support of this, 5-HT$_{2c}$ agonist action is similar to the effect of light pulses on circadian rhythmicity (i.e. on c-fos measures, on melatonin secretion and temperature rhythmicity) (176). Furthermore, altered daily profiles of serotonin in the SCN have been observed in hamsters with altered glucose tolerance pointing to a link between SCN serotonin and glucose metabolism (177). Future studies should address how and which serotonergic receptors in the SCN contribute to diurnal variation in glucose metabolism.

We here thus provide a novel hypothesis in which serotonin plays a major role in chronodisruption and metabolic disorders in conditions of misalignment. We hypothesize that in addition to the ARC and IGL the 5-HT projections from the raphe nuclei to the SCN, ARC and IGL play an important role in providing non-photic metabolic feedback to the SCN. A mismatch between the non-photic metabolic feedback received from the raphe and the photic information received from the retina by the SCN is an important trigger for chronodisruption and metabolic disease (Fig 2).
Figure 2. Proposed schedule of how nutrient and metabolic feedback are involved in proper alignment of rhythms in glucose metabolism with the environmental light/dark cycle in nocturnal rodent. A) When eating at the appropriate time of the day animals eat when it is dark a period in which the retina signals the absence of light to the SCN (dashed red line); At the same time, food intake and the related activity activates the raphe nuclei, which feeds back to ARC, IGL and SCN (yellow solid line). The signals from the raphe nuclei, IGL and ARC signal darkness to the SCN (green lines (dashed from the periphery and solid from brain areas)). B) When eating in the light period, which is an inappropriate time for nocturnal animal to eat, the retina signals 'light' to the SCN, whereas the behavioral and metabolic information from the raphe nuclei signals 'darkness' to the SCN. ARC = arcuate nucleus; SCN = suprachiasmatic nucleus; IGL = intergeniculate leaflet; 5-HT$_{2c}$ = serotonin receptor; NPY = neuropeptide Y; GABA = gamma-Aminobutyric acid ; PACAP = Pituitary adenylate cyclase-activating peptide. Green dashed line depicts the metabolic feedback from periphery; green solid lines depict metabolic feedback via neuronal projections. Yellow lines depict serotonergic projections and red lines from retina.
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