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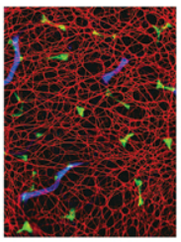

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# The effects of time-restricted feeding on lipid metabolism and adiposity

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**Maintaining natural feeding rhythms with time-restricted feeding (TRF), without altering nutritional intake, prevents and reverses diet-induced obesity (DIO) and its associated metabolic disorders in mice. TRF has a direct effect on animal adiposity, causes an alteration of adipokine signaling, and diminishes white adipose tissue inflammation. Many genes involved in lipid metabolism are normally circadian, but their expression is perturbed with DIO; TRF restores their cyclical expression. One mechanism through which TRF could affect host metabolism is by altering the gut microbiome. Changes in the gut microbiome are coupled with an altered stool bile acid profile. Hence, TRF could affect lipid metabolism by altering bile acid signaling. TRF introduces many new possibilities in treating obesity and its associated metabolic disorders. However, further studies are needed to show whether these physiological findings in mice translate to humans.**

**Keywords:** circadian rhythm, diabesity, diabetes, dyslipidemia, dysmetabolism, fasting, metabolism, steatohepatitis, steatosis

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Over the last decade, studies in mice and in humans have shown that the disruption of circadian rhythms contributes significantly to obesity.[1,2] Strong evidence from recent publications shows that the preservation of natural feeding rhythms can prevent and reverse obesity and dysmetabolism associated with diet-induced obesity (DIO) in mice.[3-6] In this commentary, we discuss the physiological effects of time-restricted feeding (TRF), including its effects on adipose tissue, adipokine signaling, lipid metabolism, gut microbiome, and stool metabolome. Furthermore, we speculate on the potential role it can play in the treatment of diabesity in human populations.

## Time-Restricted Feeding (TRF) Prevents and Reverses Obesity

Numerous metabolic genes show a diurnal pattern of expression driven by both the circadian clock and feeding/fasting cycles.[7] When fed a normal chow diet *ad libitum*, mice consume about 80% of their calories during the dark/active phase. However, when the diet is changed to one enriched in fat (high-fat diet, HFD) as in the DIO model, the timing of their caloric intake changes. DIO mice spread their caloric intake throughout the day, eventually leading to a 50/50 distribution of the food consumption between the dark/active and the light/inactive phase.[3,4,8] DIO leads to the development of obesity and its associated metabolic disorders such as hepatic steatosis, insulin and leptin resistance, and dyslipidemia.[3-5,8,9] Furthermore, the change in the feeding pattern, where calories are consumed at suboptimal times, leads to the dampening, and even the obliteration of the cyclical variation of many metabolic genes in the DIO model.[4]

The consequences of the altered feeding behavior in DIO mice, in particular the increased food consumption during the light/inactive phase, was unclear. TRF

**Table 1.** Physiological Effects of Feeding a High-Fat Diet between Diet Induced Obesity (*ad libitum* access to food) and Time Restricted Feeding (8–12 hour access to food during active phase)

| | Diet Induced Obesity* | Time Restricted Feeding* |
|---|---|---|
| Body weight | Greatly Increased | Same |
| Activity | Same | Same |
| Calories consumed | Same or Slightly greater | Same |
| Diurnal feeding pattern | Dampened | Strengthened (as part of protocol) |
| Whole body energy expenditure ($VO_2$) | Same | Increased |
| Diurnal rhythms of Circadian Oscillators (e.g., Per2, Bmal1, Rev-erbα) | Dampened | Same |
| Insulin sensitivity | Decreased | Same |
| Leptin sensitivity | Decreased | Same |
| Motor coordination | Decreased | Increased |
| Body composition from fat (Adiposity) | Increased | Same |
| Adipose tissue | Hypertrophied | Same |
| Macrophage infiltration of white adipose tissue | Present | Absent |
| Pro-inflammatory cytokines in white adipose tissue | Greatly Increased | Same |
| Hepatic steatosis | Present | Absent |
| Brown adipose tissue steatosis | Present | Absent |
| Fatty acid synthase activity | Slightly Increased | Decreased |
| Hepatic unsaturated fats | Greatly Increased | Mildly Increased |
| Stool bile acids | Same | Increased |
| Serum bile acids | Decreased diversity | Increased diversity |

Note: * When compared to mice fed a normal chow diet *ad libitum*.

can be used to preserve a more natural feeding rhythm while also maintaining the fasting state in the light/inactive phase. With TRF, feeding is consolidated to the dark/active phase and there is no access to food during the light/inactive phase. Limiting access to a HFD for 8–12 hour in the dark/active phase, does not reduce caloric intake nor the level of activity compared to that of DIO mice.[3,4] Nevertheless, TRF protects mice from developing obesity and its associated comorbidities (**Table 1**).[3-5] Furthermore, TRF restores the circadian expression of many metabolic genes to the levels observed in a normal chow fed mice.[4] Our most recent studies show that TRF can reverse obesity in DIO.[3] Furthermore, it prevents the adverse metabolic consequences of other nutritional challenges such as a high-fructose diet, or a high-sucrose/high-fat diet.[3]

## TRF Affects Adiposity and Adipokine Levels, Adipose Tissue Inflammation, and Lipid Metabolism

For mice receiving a HFD, those on TRF are leaner than mice fed *ad libitum* and almost indistinguishable from the control mice fed a normal chow diet. The body composition analysis of TRF mice reveals that they have less body fat than their DIO counterparts.[3,4] Accordingly, adiponectin and leptin were increased and decreased, respectively, in TRF mice.[3,4] The observed decrease in fat was generalized to the whole body, affecting both canonical and non-canonical fat storing organs (e.g. liver, brown adipose tissue). In particular, adipocytes of the white adipose tissue (WAT) were much smaller in TRF mice. Furthermore, the brown adipose tissue (BAT), was protected from the "whitening" that usually occurs in DIO mice where large unicolor droplets resembling white adipocytes are observed.[4]

Hepatic lipid metabolism has been analyzed extensively in TRF and DIO mice. TRF mice have reduced expression of fatty acid synthase (Fasn), a key lipogenic gene that is controlled by the transcriptional repressor Rev-erbα, which is also a circadian oscillator component.[4,10] In addition, TRF mice have reduced expression of peroxisome proliferator-activated receptor gamma (Pparγ), hence diminishing Pparγ-driven lipogenic gene expression, and its targets including stearoyl coA desaturase 1 (Scd1, an enzyme mediating fatty acid desaturation) and fatty acid elongase (Elov5). These changes coincide with significant decline in hepatic unsaturated fatty acids.[4]

Adipocyte hypertrophy in DIO is often accompanied with the accumulation of pro-inflammatory macrophages in the tissue and the development of an inflammatory environment.[3,4] Both histological examinations of the tissue and quantification of pro-inflammatory cytokines revealed an absence of inflammation in the adipose tissue of TRF mice.[3] Reduced inflammation in both WAT and BAT likely preserves their proper functioning, allowing appropriate trafficking of fat through the WAT and adequate energy burning in the BAT, thus maintaining whole-body lipid homeostasis.

The reduction of fat accumulation in adipose tissue of TRF mice could be due to reduced synthesis of fatty acids or increased fat oxidation. mRNA quantification of enzymes involved in lipid metabolism revealed that the expression of both synthesis and degradation enzymes increased in the WAT of TRF mice compared to their *ad libitum* counterparts, suggesting a higher turnover of fat in the tissue.[3] This plasticity of the adipose tissue is essential in the regulation of lipid homeostasis.

Indeed, adipose tissue regulates lipids trafficking by switching from fat storing in the anabolic state to fat release for subsequent fat oxidation in peripheral tissue in times of energy demand.

## TRF Alters Gut Microbiome

Another potential mechanism through which TRF can affect host metabolism is by altering the gut microbiome to one that is less obesogenic. Over the last decade, studies have uncovered profound changes in the composition and metabolic contribution of gastrointestinal microflora in the obese.[11-13] However the mechanism underlying the microbiome's contribution to obesity remains unclear. Earlier studies suggest that DIO causes a dysbiosis that could affect host metabolism by altering bile acid (BA) signaling, disrupting intestinal homeostasis, altering nutritional absorption, and/or activating the gut inflammatory cascade.[14-17] However, most gut microbiome studies in mouse models either alter the nutritional quality of the diet or utilize transgenic strains. These are potential factors that could obscure specific changes in the microflora that may be protective against obesity and metabolic diseases, thereby hindering our ability to find specific mechanisms that explain how dysbiosis contributes to obesity. TRF provides an ideal backdrop to study intestinal microflora since the factors that have confounded previous studies do not apply to this model.

Measuring the cecal gut microbiome at different time points in mice fed a normal chow diet reveals cyclical variations in many members of the microflora.[5] This was apparent at the phylum level with Firmicutes levels rising during the dark/active phase when the animal feeds, and decreasing during the light/inactive phase with relative fasting. Likewise, the Bacteriodetes species and Verrucomicrobia both increased during the light/inactive phase and decreased during the dark/active phase. In DIO mice, where caloric intake is spread throughout the day and night, these cyclical variations observed at the phylum level were obliterated, with Firmicutes phylum dominating the gut microbiome at all of the time points. The TRF protocol did not restore the cyclical variations at the level of the phylum.[5]

Nevertheless, TRF did restore cyclical variation in many families of bacteria that are thought to be involved in metabolism.[5] For example, TRF restored cyclical variation in the Lactobacillus family, which is also cyclical in normal chow mice, but not in DIO mice. Several Lactobacillus species have been associated with diabesity.[15,18-21] Members of the Lactobacillus species express bile salt hydrolases which conjugate gut luminal BAs and can affect BA signaling.[15] In addition, TRF restored the Ruminococcacea family, including those of the genus Oscillibacter, which are hypothesized to be protective against the metabolic consequences of obesity.[21]

Previous studies have suggested that the Firmicutes phyla was a contributor to obesity, and that a higher proportion of Firmicutes species in the gut microbiome corresponds to increased adiposity. However, measuring the microbiome at multiple time points in normal mice, and in the TRF mice shows that the level of Firmicutes species is related to the diet and feeding pattern, rather than obesity or dysmetabolism itself. This is supported by previous literature that showed that the Firmicutes phyla, as a whole, is not obesogenic and that it can change within 24 hours after a change in diet.[22-24] Furthermore, a low gut microbiome α-diversity (i.e. the types and relative amounts of species within a sample) was also hypothesized to contribute to obesity. However, when the α-diversity was averaged between all time points, there was no difference among normal chow *ad libitum*, TRF, and DIO mice. Fluctuations in α-diversity were related to diet and feeding time as opposed to the metabolic phenotype.[5]

## TRF Alters Stool Metabolome

The alterations observed in the gut microbiome of the TRF mice corresponded to shifts observed in the stool metabolome.[5] These changes were observed mostly in compounds that only gut microbiome can affect: (1) breakdown products of complex sugars and (2) BAs. These changes in the stool metabolome could hint at the mechanisms through which the gut microbiome affects host metabolism.

One potential manner the gut microbiome can affect host metabolism is that dysbiosis affects the host's nutritional absorption. In particular, the digestion of complex sugars such as hemicellulose can only be done with the aide of gut microflora, since there are no innate host enzymes that can break them down. TRF mice excreted far more breakdown products of hemicellulose (i.e., xylose and galactose), suggesting that the fermentation occurs in a region of the gut where they cannot be easily absorbed (e.g., distal colon). However, DIO mice excreted far less xylose and galactose suggesting that these animals are more efficient in absorbing them.

## TRF and Bile Acids

TRF fed mice had significant changes in primary and secondary BA levels and composition in multiple compartments (i.e. the serum, the liver and the feces) compared to DIO and mice fed normal chow *ad libitum*.[3-5] Primary BAs are produced by the liver and released during meals to facilitate triglyceride and cholesterol absorption. In the gut, they are modified by gut microbiota that express a variety of deconjugation, dehydrogenation, and dehydroxylation enzymes, leading to the formation of secondary BAs.[25] The chemical diversity of BAs is vast and properties of individual BAs only recently being understood. Aside from their essential role in fat absorption, they act as signaling molecules, through interactions with receptors including farnesoid X receptor α (FXRα) or the G protein-coupled BA receptor 1 (TGR5). In hepatocytes, FXRα signaling modulates BA, lipid, cholesterol and glucose metabolism.[26-28] Hence, BAs could act as general metabolic integrators and an agent for gut-liver signaling. Different BAs can act as either agonist or antagonist of the same receptor. For example, hydrophobic chenodeoxycholic acid is the most potent FXRα agonist while hydrophilic muricholic acid has been shown to be an FXRα antagonist.[29]

The luminal BA profile and diversity is affected by the gut microbiome. This is a

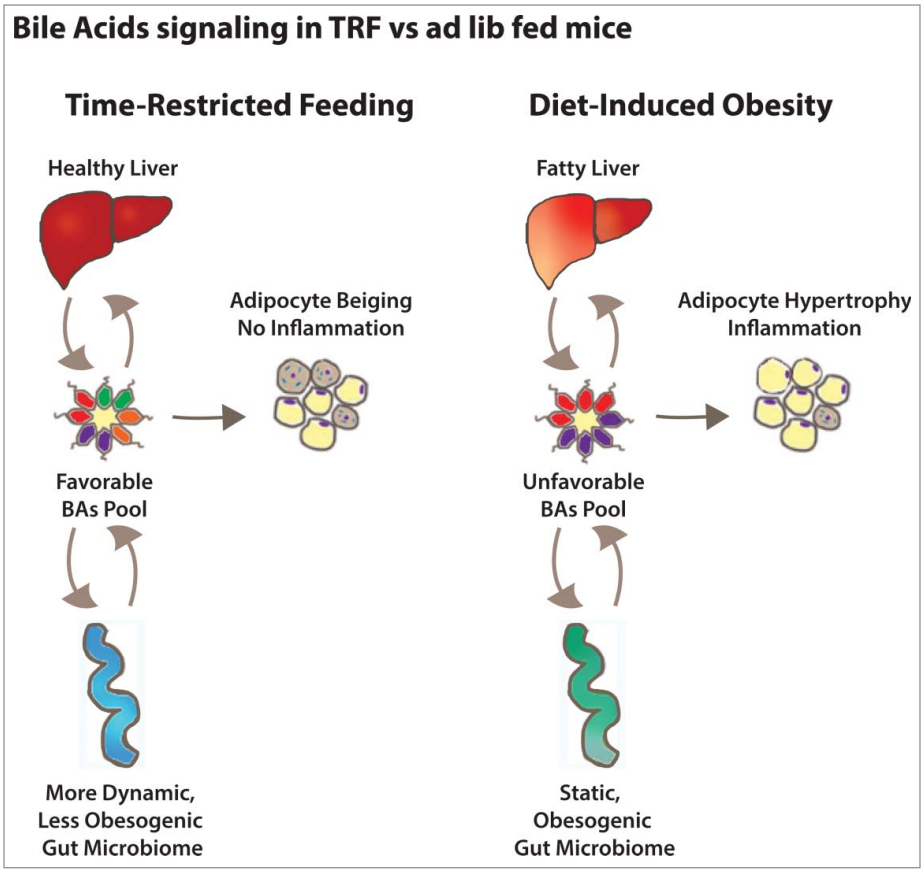


**Figure 1.** Bile acid signaling in mice fed a high-fat diet *ad libitum* or time-restricted feeding. The composition of the total bile acid pool is determined by the action of the liver (synthesis), the gut (reabsorption and excretion) and the gut microbiota (bile acids modification). Bile acids feedback on the liver and the gut regulate bile acids homeostasis and also influence their metabolic functions. They also act on the adipose tissue to regulate fat storage and utilization.

means by which the gut microbiome can affect host metabolism. For example, Lactobacillus species increase FXRα activity by conjugating tauro-β muricholic acid, an FXRα antagonist.[15] Hence different amount of Lactobacillus species in the TRF and DIO gut microbiome could mediate their affects through altered BA signaling. TRF mice have stool that is highly enriched in both primary and secondary BAs.[5]

Primary BA composition in the liver and serum are quite different between TRF and DIO mice.[3,4] For instance, cholate is more abundant in the serum of DIO mice than TRF. These proportions were inverted in the liver and feces where cholate levels are higher in TRF than DIO mice. Cholate can induce the expression of key genes involved in energy expenditure in the BAT by activating TGR5. Increased energy expenditure can lead to weight loss and fat reduction by increasing fat burning.

In sum, the diversity of the BA pool can mediate the effects of TRF. The ratio of certain BAs, some of which are FXRα agonists and antagonists (e.g. chenodeoxycholic acid v. muricholic acid v. tauro-β muricholic acid) could affect host lipid metabolism. These pathways are quite altered in TRF and DIO mice and could explain how the former's benefits are mediated (**Fig. 1**).

## TRF and Treating Obesity in Humans

More than one third (34.9%) of the US adult population is obese,[30] and it is estimated to cost the healthcare system $147 billion dollars annually.[31] Obesity is associated with multiple morbidities including diabetes, heart disease and cancer. However, there is a lack of effective, sustainable, non-surgical treatments of obesity.[32] Although light:dark cycle is known to affect the activity of the central clock, current research has shown daily cycle of eating-fasting rhythm in humans is a major determinant of daily metabolic rhythms.[7] Multiple studies have shown shift-workers are particularly susceptible to metabolic syndrome and obesity.[33] Even brief disturbances in sleep and feeding cycle in healthy individuals can affect insulin sensitivity.[34] Furthermore, after controlling for diet and lifestyle, an aberrant eating pattern over the course of a 24 hour day such as late night caloric intake, is a significant risk factor for developing coronary heart disease (CHD) by increasing CHD risk by as much as 55%.[35] Hence, TRF introduces multiple potential therapeutic methods to address this growing healthcare problem.

Part of the appeal of TRF is that it works with multiple dietary challenges (e.g., high-fat diet, high-fructose diet). Hence the behavioral intervention to treat obesity could be for patients to restrict the time that they feed, which may be easier to adopt than monitoring their caloric or macronutrient intake. Furthermore, individuals with a lower socioeconomic status, and disadvantaged minorities (e.g. Hispanics, Native Americans) are especially vulnerable to obesity and its associated metabolic disorders. These individuals are more likely to live in food deserts, where access to fresh and nutritious food remains poor. Hence, TRF may be an easier behavioral modification for these particular at-risk populations.

TRF has also identified potential obesity-protective bacteria (e.g., Oscillibacter, Ruminococcacea) which can be administered with probiotics or promoted with prebiotics as a potential treatment. Causing a more dynamic gut microbiome with timed antibiotics that are highly specific and non-absorbable could help reduce obesogenic bacteria (e.g. in the Lactobacillus family). Further studies of the metatranscriptome (i.e., the genes transcribed at a given time by the gut microflora) can help develop therapies that induce

obesity-protective bacterial genes, or silence obesogenic bacterial genes. Investigations are already underway to evaluate the role of transgenic bacteria with obesity-protective genes. Preliminary studies with fecal transplantation have been promising, further signifying that targeting gut microbiome to treat obesity and metabolic disease is an appropriate therapeutic strategy that should be investigated more thoroughly.

Potential therapies also exist in making the expression of metabolic genes more robustly cyclical with higher amplitude fluctuations. Though circadian fluctuations and their effects on metabolism have been thoroughly investigated in murine models, they remain poorly understood in primates, including humans. Nevertheless, lab-induced jet lag, as well as long-standing evidence from shift-workers show the negative consequences of discombobulating the normal circadian metabolic machinery.

One way that cyclical changes in the host metabolism can be induced is with BA mimetics that target FXRα and TGR5 receptors. These compounds are currently being actively investigated by multiple pharmaceutical companies and newly discovered ones have received tremendous press from scientific and lay audience.[36] Other compounds that signal feeding from gut to liver or gut to brain that can affect metabolism or appetite, respectively, are also actively being investigated.

Still, TRF tests in humans are preliminary and its effects in a clinical population, at this time, remains purely speculative. Currently there are multiple active clinical studies investigating whether its effects hold in particular vulnerable populations. With further studies, it will be interesting to see if this fascinating metabolic phenomenon translates to humans.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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