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Effects of reciprocal interactions between various dietary fats and circadian phases on postprandial hyperlipidemia in rats

Basri Satılmış[a], Başak Kayhan[b] and Tayfun Güldür[c]

[a]Hepatology Research Laboratory, Liver Transplantation Institute, İnönü University, Malatya, Turkey; [b]Faculty of Medicine, Department of Medical Biology and Genetics, İnönü University, Malatya, Turkey; [c]Faculty of Medicine, Department of Medical Biochemistry, İnönü University, Malatya, Turkey

ABSTRACT

Expression levels of various intestinal proteins involved in postprandial lipoprotein assembly as well as plasma triglyceride concentration exhibit daily oscillations indicating circadian control. The length of the carbon chain and degree and position of unsaturation of fatty acids influence triglyceride secretion by the enterocytes. To this end, effects of reciprocal interactions of various single fats/oil (olive oil, fish oil or butter) gavaging either in active or passive phase were investigated in rats. Fat/oil gavaged in the active phase of circadian rhythm resulted in higher postprandial serum triglyceride levels compared to that in the passive phase. Moreover, olive oil led to higher MTP activity and apo B-48 gene expression, while fish oil gavaging caused more prominent apo B-48 and MTP gene expression when they were given in the passive phase. The present results indicate that circadian time at which fat or oil gavaged once might exert influence on postprandial lipoprotein synthesis/assembly.

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KEYWORDS
Circadian rhythms; dietary fats; chylomicron; hyperlipidemia; microsomal triglyceride transfer protein

Introduction

Circadian rhythm is defined as rhythmic oscillations in physiological processes which enable living organisms to expect upcoming changes in the environment and to make necessary arrangements accordingly (Güldür & Otlu 2017). It is believed that there are two kinds of clock functioning in the body. The master clock resides in hypothalamus oscillating in conjunction with light/dark cycle, whereas peripheral clocks occur in peripheral tissues and influenced by other environmental factors such as feeding regimens. The rhythmic expression and activity of the metabolic pathways are mainly attributed to the coordinated expression of clock genes (Clock, Bmal1, Per2, Per 1, Per3, Cry1 and Cry2) (Froy & Miskin 2007). Mutual interactions among clock, metabolism, and food appear to exist in mammals (Oike et al. 2014). Food intake itself can also generate entraining signals for peripheral clocks. These signals may involve food metabolites, hormones that are secreted upon feeding and fasting, and/or the intracellular redox state (Schmutz et al. 2012).The current studies indicate that consumption of food at inappropriate times lead to metabolic dysfunctions due to disruption

CONTACT Basri Satılmış basri.satilmis@inonu.edu.tr

of circadian rhythm which results in diabetes, obesity, and heart diseases (Schroeder & Colwell 2013).

During the postprandial state, the dietary lipid is transported from the intestine to peripheral tissues by plasma lipoproteins called chylomicrons. Postprandial lipids and lipoproteins have been associated with the presence of cardiovascular disease in a large number of case control studies (Nordestgaard et al. 2007; Lindman et al. 2010). There is considerable theoretical and experimental evidence to support an atherogenic role for the smaller, triglyceride (TG)-rich postprandial lipoproteins. The postprandial plasma levels of small chylomicron remnants (Sf 20–60 apolipoprotein B-48) were found to relate distinctly to the rate of progression of coronary lesions (Mero et al. 2000).

Expression levels of various proteins involved in chylomicron synthesis and assembly including apo B-48, apo A-IV, and MTP exhibit daily oscillations indicating that they are under circadian control (Hussain & Pan 2012). MTP has an important role in lipoprotein (chylomicron) packaging and its activity oscillates in a time-dependent manner in enterocytes. Intestinal gene and protein expression of apo A-IV in ad libitum fed rats was reported to vary in response to the time of day. Apo B-48 gene expression responds to lipid absorption and also shows diurnal changes in their expression (Pan & Hussain 2009). HNF-4α, a nuclear transcription factor, plays a role in the regulation of intestinal apo A-IV expression levels in enterocytes in response to lipid absorption (Carriere et al. 2005). Furthermore, the binding of HNF-4α to the MTP promoter is substantial for MTP expression (Hayhurst et al. 2001). Therefore, HNF-4a plays a key role in the postprandial secretion of TG. Clock and clock-controlled genes play an important role in intestinal gene expression involved in nutrient absorption (Hussain & Pan 2012). Plasma total TG and lipoproteins also exhibit time of day-dependent oscillations. These oscillations are the result of not only rhythms in food intake but also rhythms in lipoprotein-associated TG hydrolysis by lipoprotein lipase (LPL) which appears to be under circadian clock control (Arasaradnam et al. 2002).

The total amount of fat, the length of the carbon chain, and degree and position of unsaturation of fatty acids seem to influence TG secretion rate by enterocytes and their atherogenic effects in the postprandial state (Lopez-Miranda et al. 2007). It has been reported that influences of dietary fats/oils on postprandial inflammatory changes associated with atherosclerosis might depend not only on their fatty acid compositions but also on their ingestion times (Otlu et al. 2016).

To this end, it is aimed, in the current work, to examine in rats the effects of reciprocal interactions of various fat/oil gavaging including butter (rich in saturated fatty acids), olive oil (rich in monounsaturated fatty acids), or fish oil (rich in ω-3 polyunsaturated fatty acids) and their administration times either in light or dark phases on postprandial TG levels and the expression levels of proteins involved in chylomicron synthesis and assembly. Single fasting and consequent single gavaging were conducted in order to avoid or to minimize any phase shifting effect of changing time of food availability.

Materials and methods

Animals and experimental design

All the animal procedures were performed in accordance with the İnönü University Ethical Committee of Experimental Animals and in compliance with the care and use of laboratory

animals. Male Wistar rats with 300–350 g body weight obtained from Experimental Animal Unit of İnönü University were used throughout the study. They were maintained under constant laboratory conditions of 12 h light and 12 h dark cycles and temperature and allowed access to standard pellet diet and water ad libitum.

Investigation of postprandial lipoprotein assembly/synthesis following various fat/oil gavaging at different zeitgeber time points

Rats were divided into 16 groups each containing 4–5 rats. Each type of fat/oil application included four groups with gavaging at different zeitgeber time (ZT) (i.e. at ZT0, ZT6, ZT12, or ZT18). Control groups were also allocated to each of the gavaging time points. Control groups received saline solution. Following 10 h fasting, rats were given 1 mL of different fat or oils including butter (rich in saturated fatty acids), olive oil (rich in monounsaturated fatty acids), and fish oil (rich in ω-3 polyunsaturated fatty acids) by single gavaging at ZT0 (beginning of passive phase), ZT6 (halfway through the passive phase), ZT12 (beginning of active phase), or ZT18 (halfway through the active phase). Four hours after gavaging, the rats were euthanized and blood and small intestine samples were collected. The small intestine samples were divided into 5-cm segments starting from the duodenum. Serum and small intestine samples were kept at −20 and −80 °C, respectively, until analyzed. First, 5-cm segment of small intestine was analyzed for MTP activity, whereas second 5-cm segment for apo B-48, apo A-IV, HNF-4α, and MTP mRNA quantifications.

Comparison of postprandial hypertriacylglyceridemia between various fat/oil administration at ZT0 and ZT12

The rats were divided into eight groups, each containing 4–5 rats. Each one of the groups was allocated to one of the fat/oil administration and one ZT point. Blood samples were obtained by exsanguination one hour, two hours, or four hours after fat/oil administration at ZT0 or ZT12.

Postprandial TG measurement

Blood samples were obtained from the heart or inferior vena cava to measure postprandial TG levels. Serum TG levels were determined by EnzyChrom™ Triglyceride Assay Kit from BioAssay Systems (Hayward, CA, USA) according to the protocol provided by the manufacturer.

MTP activity assay

Small intestine samples were homogenized in buffer containing 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 2% protease inhibitor cocktail (Sigma–Aldrich). MTP activity was measured by an MTP assay kit (Roar Biomedical, New York, NY, USA) according to manufacturer' s instructions. Hundred micrograms proteins of intestinal homogenates were incubated with 4 µL donor solution and 4 µL acceptor solution in a total volume of 200 µL of homogenization buffer. Fluorescence was measured by BioTek Synergy H1 m (Winooski, VT, USA) at 485 and 515 nm excitation and emission wavelengths, respectively, at 37 °C.

Intestinal gene expressions

Total RNA was isolated from small intestine tissue using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). RNA was quantified measuring the absorbance at 260 nm (A_{260}) and RNA purity was determined by the ratio of A_{260}/A_{280} using MaestroNano spectrophotometer (Maestrogen, Las Vegas, NV, USA). Total RNA from all samples was reverse transcribed to cDNA using RT2 HT First Strand Kit (Qiagen, Maryland, MD, USA) on a thermal cycler (SensoQuest, Göttingen, Germany). Quantitative real-time PCR was conducted on Rotor-Gene Q (Qiagen, Hilden, Germany) using RT2 SYBR Green ROX Fast Master Mix (Qiagen, Maryland, MD, USA) and specific primers for GAPDH (Cat No: PPR06557B, RefSeq Accession No: NM_017008), ApoA-IV (Cat No: PPR46160F, RefSeq Accession No: NM_012737), ApoB (Cat No: PPR48645C, RefSeq Accession No: NM_019287), MTP (Cat No: PPR55426A, RefSeq Accession No: NM_001107727), and HNF-4α (Cat No: PPR49773A, RefSeq Accession No: NM_022180) were obtained from Qiagen. PCR cycling conditions were initial denaturation at 95 °C for 10 min and followed by 40 cycles of denaturation at 95 °C for 10 s, and annealing at 60 °C for 30 s. During thermal cycling, emission from each sample was recorded and Rotor-Gene Q software processed the raw fluorescence data to produce threshold cycle (Ct) value. The housekeeping gene GAPDH was used as a reference gene for normalization of data. Relative fold changes in gene expressions were calculated using the $2^{-\Delta\Delta Ct}$ method. To obtain a ΔCt value, the Ct value of GAPDH was subtracted from that of the gene of interest. Then, the ΔΔCt value was calculated by subtracting the ΔCt value of control group from the ΔCt value of experimental groups. Finally, fold change was calculated by taking 2 to the power of minus ΔΔCt value.

Analysis of retinyl palmitate concentration

Retinyl palmitate (RP) concentrations in blood samples collected 1, 2, and 4 h after gavaging with fat/oils containing 100,000 IU of RP were analyzed by HPLC. Two hundred microliters of serum samples were denaturated with an equal volume of absolute ethanol. Then, 500 µL of cold n-hexane was added and the solution was vortexed for 5 min and centrifuged at 16,000 × *g* for 5 min at 4 °C. Upper hexane phase was transferred into a tube and hexane extraction process was repeated. Combined hexane extracts were evaporated to dryness under a gentle stream of nitrogen. The dried residue was resuspended in cold ethanol, vortexed and injected into the HPLC column. The chromatographic analysis of serum RP was carried out using a UniverSil C18, 250 × 4,6 mm i.d., 5 µm particle size analytical column (Fortis Technologies, Cheshire, UK) on an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was a mixture of methanol:ethanol (75:25, v/v). The flow rate was kept at 0.8 mL/min and the diode array detector was used for the quantification at 325 nm (Kand'ar et al. 2013).

Statistical analysis

Data are given as median, minimum, maximum, and interquartile ranges. Statistical differences between groups were tested with Kruskal–Wallis and Mann–Whitney *U*. Post hoc Conover multiple comparison test was performed for pairwise comparison of groups. *p* value less than 0.05 was considered as significant for all statistical analysis.

Results

Influence of various fat/oil gavaging at different ZT on postprandial lipoprotein assembly/synthesis and TG secretion

Following 10-h fasting, rats were given different fat or oils including butter, olive oil, and fish oil by gavaging at ZT0 (beginning of passive phase), ZT6 (halfway through the passive phase), ZT12 (the beginning of active phase), or ZT18 (halfway through the active phase). Blood and small intestine samples were obtained 4 h after the gavaging.

Comparison of serum TG levels between different ZT groups

Serum TG levels of rats gavaged either with olive oil or butter at ZT12 or ZT18 were found to be higher compared to those gavaged at ZT0 or ZT6. Moreover, rats were given fish oil either at ZT12 or ZT18 and also had higher serum TG levels in comparison to that gavaged at ZT6. All the fat or oils gavaged either at ZT12 or ZT18 resulted in higher serum TG levels compared to that at ZT6 and, with the exception of fish oil, to that at ZT0 (Figure 1(A)).

The present results indicate that the ZT at which fat or oils is given influences the level of postprandial hypertriglyceridemia (and, as a result, chylomicron synthesis). Fat/oil gavaging during the active phase of circadian rhythm in rats results in more TG secretion as compared to that during passive phase of the rhythm.

Comparison of serum TG levels between different fat/oils group

Olive oil administration yielded higher serum TG level compared to butter administration at all ZTs except at ZT12. Fish oil administration at ZT18 also resulted in higher serum TG level in comparison to butter gavaging (Figure 1(A)).

Intestinal MTP activities

Comparison of MTP activities of intestines between rats given different fat or oils display a similar order, that is olive oil > fish oil > butter, at each ZT points. Nevertheless, the differences were found to be insignificant. On the other hand, intestinal MTP activity was found to be significantly higher 4 h after olive oil gavaging at ZT6 compared to that of butter gavaging or control group. (Figure 1(B)).

Intestinal gene expression levels of apo B-48, apo A-IV, HNF-4α, and MTP

Apo B-48 gene expression levels of the group gavaged with fish oil or olive oil at ZT6 were found to be significantly higher compared with butter**.** Although apo B-48 gene expression levels of the groups gavaged with butter at the other ZT time points also appeared to be lower in comparison to other fat/oil groups, the difference was insignificant (Figure 2(A)). ApoA-IV and HNF-4α gene expression levels in gavaging with fish oil at ZT6 were found to be higher in comparison to olive oil and butter. However, comparison of apo A-IV and HNF-4α gene expression levels in the small intestinal tissues between neither the different fat/oils groups nor between the different ZT points produced a significant difference (Figure 2(B) and (C)). As with the analysis of intestinal MTP activity and ApoB-48 gene expression, analysis of MTP gene expression levels by PCR 4 h after fat/oil administration at various ZT points also revealed an up-regulation (activation) after fish oil gavaging at ZT6 compared to that after both olive oil or butter load at the same ZT point (Figure 2(D)).

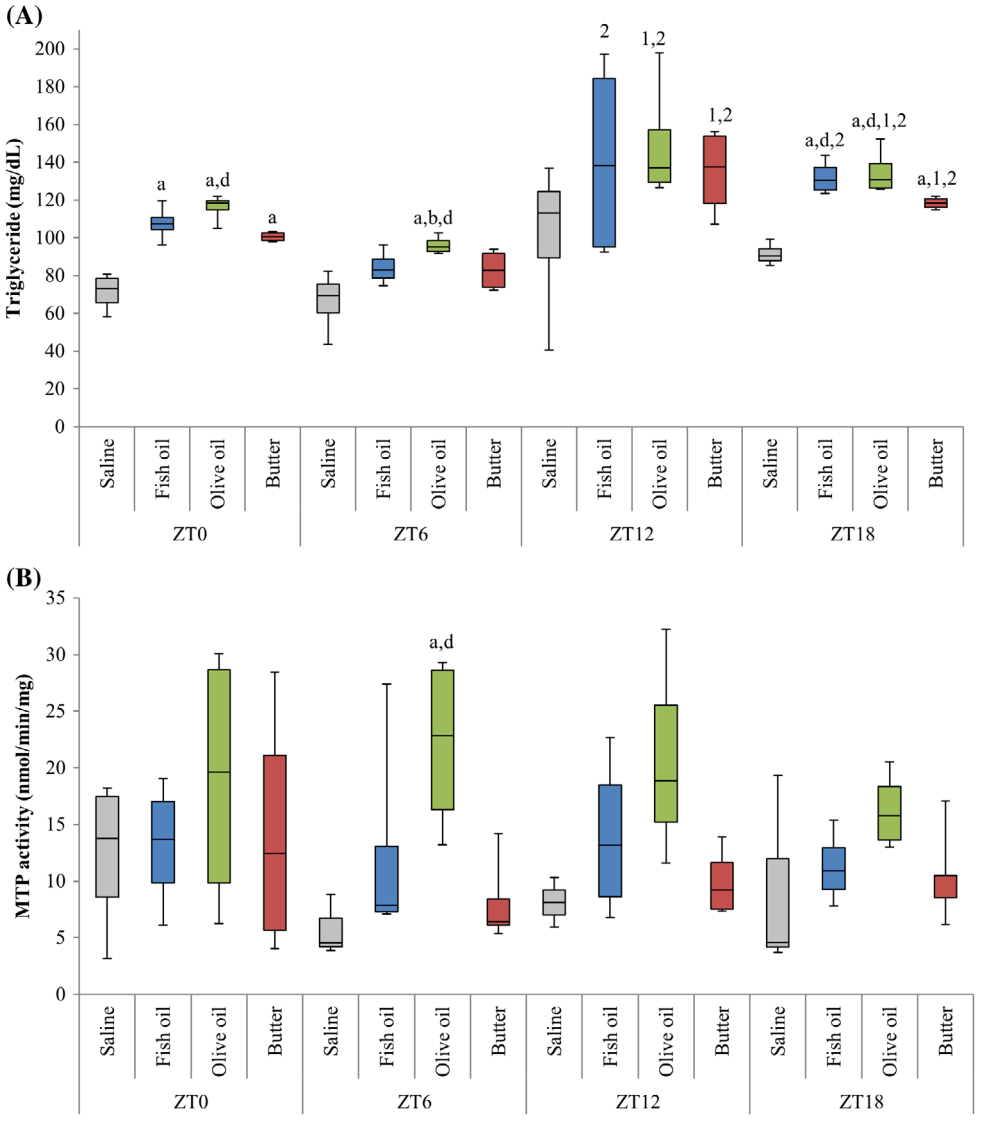

Figure 1. Comparison of the effects of different fat/oil on postprandial triglyceride levels and MTP activity at four different zeitgeber time (ZT0, ZT6, ZT12, and ZT18) points. Blood and the small intestine specimens were collected 4 h after fat/oil bolus. A: Postprandial serum triglyceride levels. B: MTP activity in the small intestine.

Notes: Data are given as median, minimum, maximum, and interquartile ranges. The horizontal line in the box represents the median, the lower and upper borders of the box are first and third quartiles, respectively, and the whiskers are minimum and maximum values. *p* values less than 0.05 were regarded as statistically significant. For a given a fat/oil, the number indicates that corresponding value differs from other ZT points (1 for ZT0, 2 for ZT6). a: statistically different compared to saline, b: compared to fish oil, c: compared to olive oil, d: compared to butter loads.

Comparison of postprandial TG levels between various fat/oils administrations at ZT0 and ZT12

Blood samples were collected by exsanguination from different groups of rats one hour, two hours, or four hours after single fat/oil gavaging either at ZT0 or ZT12.

Comparison of postprandial TG values between fat/oil administrations at ZT0 and ZT12

Single fat/oil applications at ZT12 led to higher postprandial TG response compared to that at ZT0 at each time point with the exception of 4-h postprandial TG levels after fish oil ingestion, which was also higher albeit insignificant (Figure 3(A)–(C)). The preceding data indicate

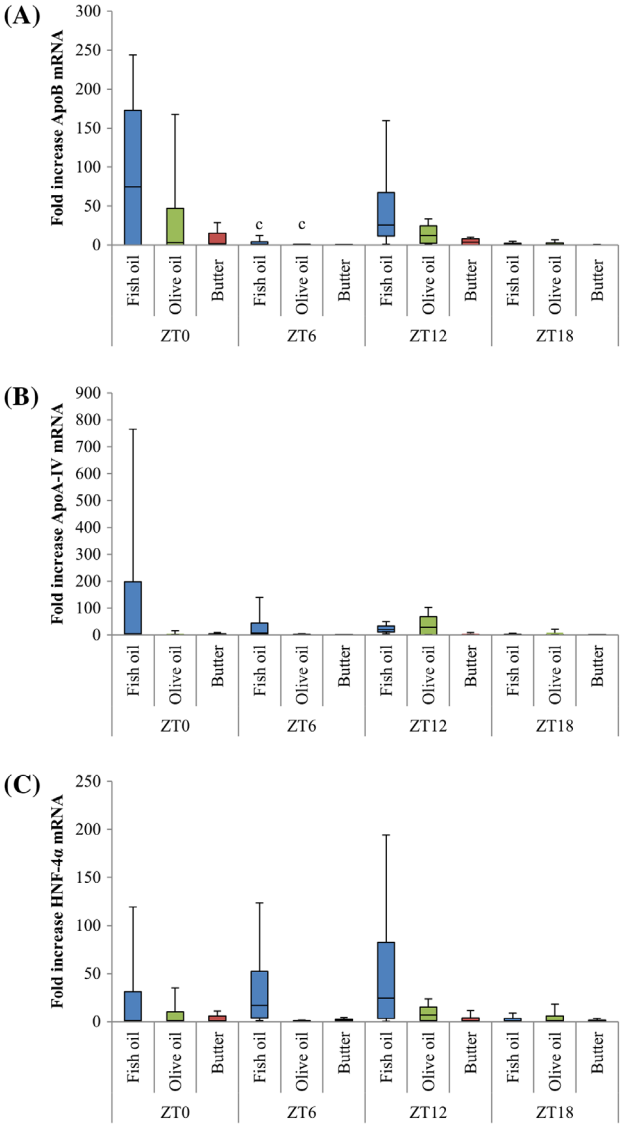

Figure 2. RT-PCR analysis of gene expression in the small intestine after different fat/oil gavaging at ZT0, ZT6, ZT12, and ZT18. A: ApoB-48 gene expression levels, B: ApoA-IV gene expression levels, C: HNF-4α gene expression levels, and D: MTP gene expression levels.

Notes: Data are given as median, minimum, maximum, and interquartile ranges. The horizontal line in the box represents the median, the lower and upper borders of the box are first and third quartiles, respectively, and the whiskers are minimum and maximum values. P values less than 0.05 were regarded as statistically significant. a: statistically different compared to fish oil, b: compared to olive oil, c: compared to butter loads.

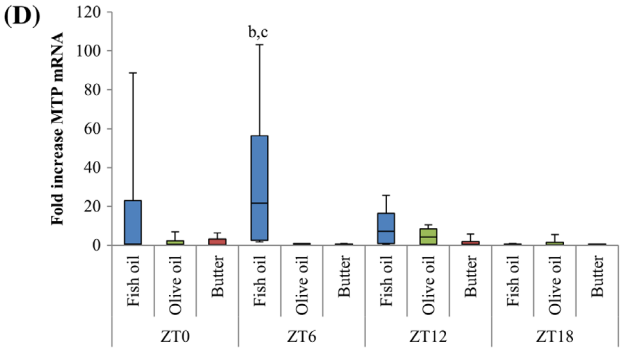

Figure 2. (*Continued*).

that single fat administration at the initiation of the active phase of circadian rhythm results in higher postprandial serum TG response compared to that at the initiation of the passive phase in the rats.

Serum RP levels following single gavaging of various fat/oils at ZT0 or ZT12

100,000 IU RP was added to fat or oil dosage before oral administration by gavaging. Since RP is packed into nascent chylomicrons after absorption from small intestines, serum RP concentrations are used as indicators of newly synthesized chylomicron concentrations especially shortly after fat/oil load. Two- and 4-h postprandial serum RP levels following either olive oil or butter gavaging at ZT0 were greater compared to that of 1-h postprandial levels of corresponding fat/oil administrations. In the case of fish oil application, only 2-h postprandial RP levels were significantly higher compared to 1-h postprandial level. The results imply that postprandial serum RP levels of fat/oils applications at ZT12 follow a similar pattern to that at ZT0 with the exception that RP levels 2 or 4 h after fish oil and 4 h after butter administrations were also found to be higher compared to the corresponding levels after 1 h albeit insignificant.

In comparison of postprandial RP levels between fat/oils applications at ZT0 and ZT12, it can be envisaged that following fat/oils applications at ZT12, serum RP levels reached a maximum mean level of approx. 2–2.5 ppm 2 h after gavaging, whereas at ZT0 the values varied between approx. 0.8–1.1 ppm. However, the differences were not significant possibly because of high variations in the data. Higher postprandial levels of serum RP at 2 h following fat/oils administrations at ZT12 compared to that at ZT0 are consistent with postprandial serum TG levels. Moreover, olive oil gavaging produced highest levels of TG as well as RP at 2 h postprandially.

Ratios of postprandial serum concentrations of TG/RP 1, 2, and 4 h after each single fat/oil administration at ZT0 or ZT12 can be compared in Table 1. In general, the ratios were found to be higher after fat/oil gavaging at ZT0 in comparison to that at ZT12. Moreover, it can be seen from the table 1 that the ratios decreased 2 h after gavaging compared to postprandial 1 h possibly because of hydrolysis of TG-rich postprandial lipoproteins by LPL. However, the ratios were slightly increased 4 h after gavaging compared to that of 2 h after gavaging probably due to secretion of RP via TG-rich VLDL particles following uptake of postprandial chylomicron remnants by the liver. Olive oil gavaging at ZT12 produced

statistically significant difference in ratio of TG/RP after 2 h compared to that of 1 h. Nevertheless, with the exception of olive oil gavaging at ZT12, neither between TG/RP ratios of postprandial hours from each of fat/oil administrations nor between different fat/oils produced statistically significant differences.

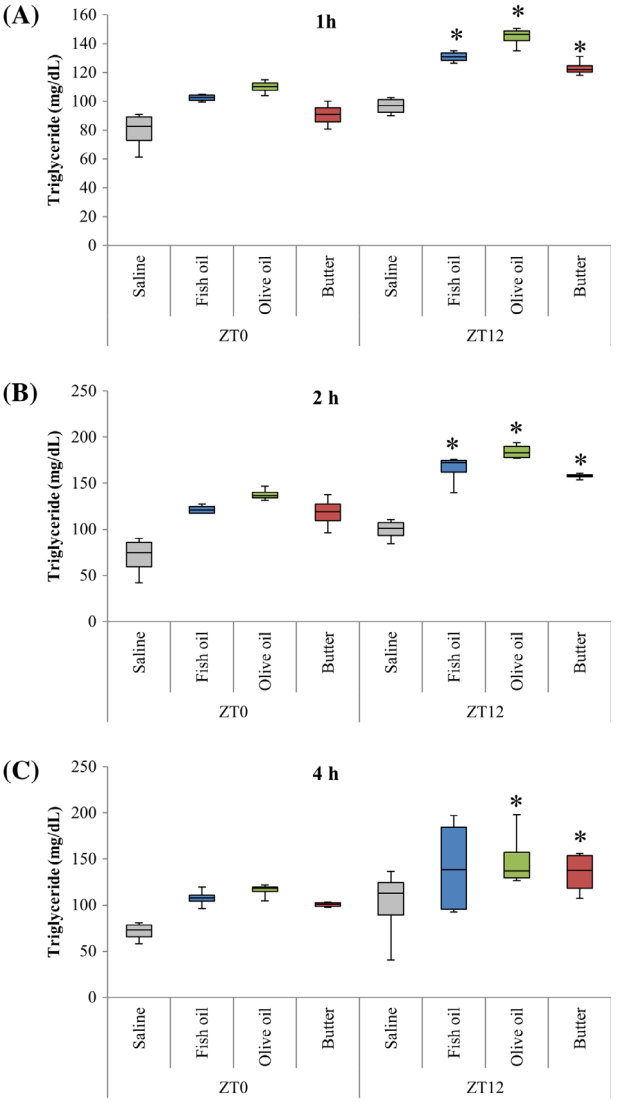

Figure 3. Comparison of the effects of different fat/oil gavaged at ZT0 or ZT12 on postprandial triglyceride and retinyl palmitate levels. Postprandial triglyceride levels 1, 2, or 4 h after fat/oil gavaging at ZT0 and ZT12 were shown in A, B, and C, respectively. Postprandial retinyl palmitate levels 1, 2, or 4 h after fat/oil gavaging at ZT0 and ZT12 were shown in D, E, and F, respectively. Notes: Data are given as median, minimum, maximum, and interquartile ranges. The horizontal line in the box represents the median, the lower and upper borders of the box are first and third quartiles, respectively, and the whiskers are minimum and maximum values. *p* values less than 0.05 were regarded as statistically significant. For a given fat/oil, asterisk (*) indicates that the corresponding value differs between ZT0 and ZT12.

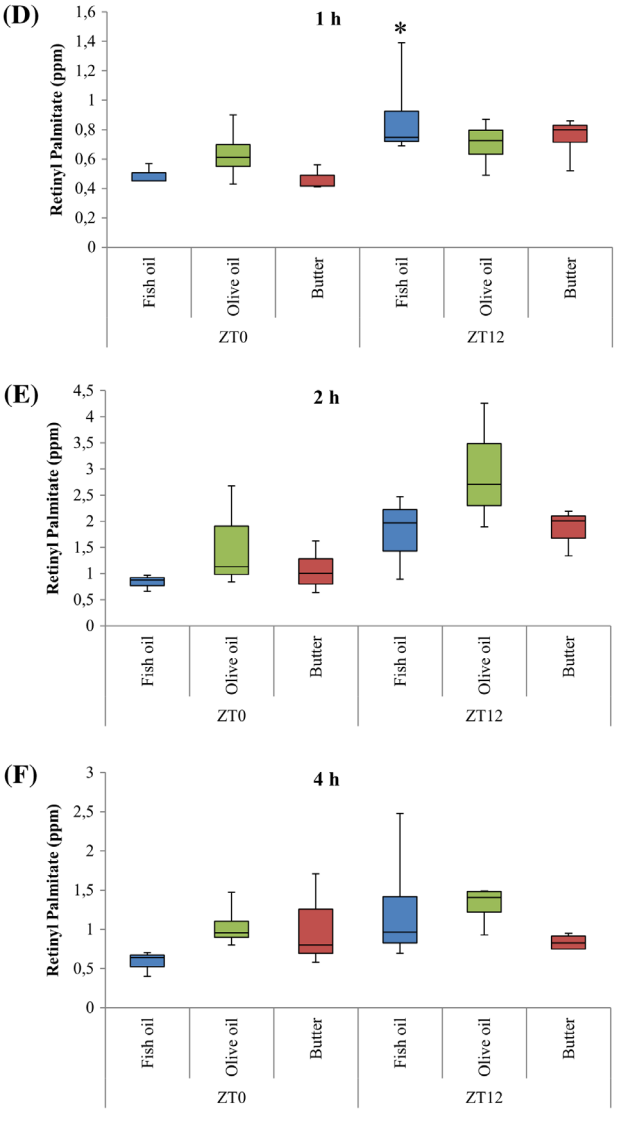

Figure 3. (*Continued*).

Table 1. Postprandial serum triglyceride to retinyl palmitate ratio 1, 2, and 4 h after oral gavage of fish oil, olive oil, or butter containing 100,000 IU retinyl palmitate at ZT0 and ZT12.

	1 h		2 h		4 h	
	ZT0	ZT12	ZT0	ZT12	ZT0	ZT12
Fish oil	226,712	175,23	137,805	87,482	167,842	143,003
Olive oil	180,598	202,121	120,582	67,688*	124,188	97,558
Butter	218,654	152,466	117,985	78,754	125,965	166,616

Notes: *p* values less than 0.05 were regarded as statistically significant. For a given fat/oil, asterisk (*) indicates that the corresponding value differs from 1-h postprandial time point at same ZT.

Discussion

Postprandial chylomicron synthesis was evaluated and compared after gavaging with butter, olive oil, or fish oil at different ZTs. In our work, the rats were starved for 10 h before gavaging fat or oil in order to promote digestion and absorption of fat/oil. However, it is well known that shifting food availability to a time different from a standard light/dark cycle causes a phase shift in some peripheral tissues (Bass 2012; Peek et al. 2012). Since animals adjust to new feeding schedule within a few days (Froy 2007; Froy & Miskin 2007), single fasting and consequent single gavaging were conducted in order to avoid the occurrence of any phase shifting effect of altered food availability.

The present results indicate that the ZT at which fat or oils administered influences levels of postprandial hypertriglyceridemia. Fat/oil gavaging either at the beginning of the active phase or at halfway through the active phase produced higher plasma TG levels compared to that at the beginning of or halfway through the passive phase in rats.

The postprandial plasma TG levels following fat/oil gavaging are associated with lipid digestion/absorption and lipoprotein (chylomicron) assembly and secretion. Multiple aspects of digestion and absorption exhibit a time of day dependence in mammals (Bray & Young 2011). Clock regulates expression of several transport proteins involved in nutrient absorption which was high at night and low during the day (Pan & Hussain 2009; Hussain & Pan 2012). Plasma total TG and lipoproteins have also been reported to exhibit time of day-dependent oscillations. When a high-fat meal is consumed at the beginning of the active phase, digestion and absorption occur swiftly followed by elevation of chylomicrons. Conversely, consumption of high-fat meal at the end of the active phase, lipid digestion and absorption are slower (Bray & Young 2011). Moreover, it was reported that cell autonomous clocks directly regulate TG metabolism, promoting TG synthesis at the end of the active/awake phases in mice (Bray et al. 2010). Taken together the preceding data, it can be envisaged that the postprandial TG levels following fat/oil gavaging are time of day dependent, being high at night (active phase) and low during the day (passive phase) which is in line with our findings. Plasma lipoproteins also show diurnal variations, they are high in the day and night in humans and rodents, respectively. Clock plays a role in the control of daily changes in plasma lipoproteins. Although the availability of food is the major determining stimuli for the mobilization chylomicrons, light and clock appear to be necessary for optimum food entrainment of plasma lipoproteins (Hussain & Pan 2012). The postprandial plasma TG levels following fat/oil gavaging are associated with lipoprotein assembly which depends on apo B-48, apo A-IV, and MTP (Pan & Hussain 2009). MTP has an important role in lipoprotein packaging. Researchers observed a significant correlation between changes in MTP expression and variations in plasma TG-rich lipoproteins (TRL) in mice (Bray & Young 2011; Hussain & Pan 2012).

MTP expression and plasma lipids were reported to undergo diurnal regulation and exhibited peaks (at 24:00) and nadirs at similar times (Pan & Hussain 2007). Additionally, in mice, MTP activity, protein, and mRNA activity were found to be high at night and low during the day (Pan & Hussain 2009; Hussain & Pan 2012). Workers, therefore, reported that light–dark cycle is required for the regulation of intestinal MTP by food (Pan & Hussain 2009).

It can be seen from our results that fat/oil gavaging in active phase produced higher plasma TG levels compared to that in passive phase; however, this was not accompanied by an increase either in MTP activity or in MTP expression in our hand. This could be due to a

single fat/oil load application. In some works, high-fat feeding continued for one week (short term studies). As a result, it can be envisaged that one fat/oil application might not be enough to see any alteration in MTP activity or expression levels. However, comparison of MTP activities of intestines between groups revealed the similar descending order as olive oil > fish oil > butter at each ZT points which accompanies the descending order plasma TG levels produced by these fat or oils. Nonetheless, the differences between the groups were not statistically significant probably owing to single fat/oil applications.

Apo A-IV synthesis and secretion are stimulated by active lipid absorption and chylomicron formation in the small intestine in rodents (Kohan et al. 2015). Intestinal gene expression of apo A-IV in ad libitum fed rats varied in response to the time of day (Shen et al. 2005). Apo A-IV mRNA and protein levels were significantly higher during the dark phase than during the light phase (Pan & Hussain 2009). However, in our study, comparison of apo A-IV gene expression levels in the small intestinal tissues among various gavaging time points of a given fat or oil caused an insignificant difference. On the other hand, fat-dependent modulation of intestinal apo A-IV gene expression was reported to be a long-term process requiring weeks to be completed (Kalogeris & Painter 2001). Stimulatory effects of fatty acids *in vitro* required a relatively long incubation time (20 h) in contrast to short periods (30 min) during which fatty acids exhibited inhibitory actions. Taken together it can be hinted that longer fat feeding period is needed in order to see the stimulatory effect of fat/oils on apo A-IV expression levels. Apo A-IV synthesis was found to vary depending on the types of fatty acids infused. No stimulation of apo A-IV synthesis and secretion was found following short chain fatty acids because of their direct secretion into the portal vein. However, monounsaturated and polyunsaturated fatty acids of n-3, n-6, and n-9 families are able to promote apo A-IV biogenesis through modulation of apo mRNA levels (Stan et al. 1999). But in our case, different fat/oil gavaging did not lead to altered apo A-IV gene expression levels probably because fat/oil feeding frequency fell short of time required for induction of apo A-IV synthesis.

Some works in mice enterocytes suggest that HNF-4α is involved in the transcriptional activation of apo A-IV by dietary lipid. Apo A-IV is also known to have binding sites for HNF-4α (Carriere et al. 2005). It can be conferred from our results that no induction HNF-4α expression occurred after fat/oil loads which agrees to the lack of apo A-IV expression in small intestines after fat/oils administrations.

It was stated that apo B-48 mRNA was constitutively expressed in the small intestine which stores apo B-48 for the assembly and secretion of chylomicrons. Apo B mRNA levels were not appreciably affected by the high-fat intraduodenal infusion (Leng et al. 2007). It was reported that during the short-term high-fat diet (HFD), intestine adapts its postprandial secretion of TRL by decreasing the number of apo B-48 containing chylomicron particles and by increasing TG synthesis and secretion. This first phase of adaptation to HFD led to the secretion of a smaller number of larger sized chylomicrons. The second phase of intestinal adaptation occurred upon longer term HFD. In this phase, intestine increases delivery efficiency by increasing numbers of postprandial apo B-48 TRL (Hernandez Vallejo et al. 2009). Moreover, in line with the preceding data, in our study, apo B-48 expression levels did not differ significantly between fat/oil loadings (except for fish oil or olive oil load at ZT6). In mice fed ad libitum and kept in 12 h LD cycle, apo B mRNA levels were reported to be high during the night and low during the day (Pan & Hussain 2009). However, we did not find any difference in terms of apo B-48 expression levels of oil/fat loads in active and passive phases.

The discrepancy might be attributed to differences in feeding regimens, i.e. ad libitum versus fat/oil gavaging.

Our work indicates no differences between intestinal activities (MTP) and expression levels of proteins taking part in postprandial lipoprotein assembly (apo B-48, apo A-IV, MTP, and HNF-4α) in passive and active phases. However, it is intriguing that in comparison to butter gavaging, olive oil led to higher MTP activity and apo B-48 gene expression levels, while fish oil gavaging gave rise to more prominent apo B-48 and MTP gene expression levels when they were given in the passive phase (at ZT6).

This can be attributed to food anticipatory activity (FAA). FAA exhibits properties of circadian control whereby organisms can predict the time of upcoming foods and activate its appetite, digestive secretions, and metabolism prior to food intake (Garaulet & Madrid 2010; Mistlberger 2011). In this way, proteins of lipoprotein assembly including MTP and apo B can become available before food (fat/oil) intake. Additionally, FAA was reported to play a major role in controlling MTP (Pan & Hussain 2007). Taking together the preceding data, it might follow that higher ApoB and MTP gene expression in the halfway through the passive phase (ZT6) can be regarded as a preparation stage for upcoming food intake and consequent higher TG/lipoprotein synthesis/assembly. Whether these results imply a preliminary sign of variation in response to gavaging at different circadian times is far from conclusive. The present results, therefore, warrants studies on long-term effects of fat/oil feeding in active or passive phases.

The influence of fatty acids on TG secretion by enterocytes differs to some extent. Studies indicate that in cells incubated with n-3 fatty acids (found in fish oil), TG synthesis was markedly inhibited and accumulation of cellular TG was decreased compared to that incubated with oleic acid (found in olive oil). In vitro study reported that fish oil lowers TG synthesis and secretion in enterocytes (Murthy et al. 1992). On the other hand, unsaturated fatty acids were found to be more potent than saturated fatty acids (found in butter) in stimulating TG synthesis and secretion of lipoproteins. Saturated fatty acids were less efficiently secreted by enterocytes than unsaturated fatty acids because they enter portal vein rather than to lymph duct (Lopez-Miranda et al. 2007). It was proposed that olive oil promoted secretion of TRLs of larger particle size than the PUFA-rich mixture (van Greevenbroek et al. 1996). In our work, efficiencies of fatty acids in promoting TG synthesis can be placed in descending order as olive oil > fish oil > butter which is in line with what have already been known.

In comparison of postprandial TG levels between various fat/oils administrations at ZT0 and ZT12, it can be inferred from the present results that fat/oil applications in active phase led to higher plasma TG levels in comparison to that in passive phase. Postprandial serum RP concentrations also followed a similar pattern. Since serum RP levels were used as an indicator of newly synthesized TG (chylomicron) concentrations, the ratio of postprandial serum concentrations of TG/RP could indicate the ratio of amounts of TG already present in enterocytes to that of newly synthesized TG. Our results indicate higher ratio of TG/RP in serum from oil/fat applications in passive phase compared to that in the active phase. The results might imply that when the fat/oil was applied in the passive phase, TGs already present in enterocytes are more readily packed into nascent lipoproteins (chylomicrons). In contrast, in the case of the active phase applications, mostly newly synthesized TGs are loaded into the lipoproteins. Nonetheless, the differences between TG/RP in active and passive phases were insignificant albeit consistent. Whether or not the time of dependence of multiple aspects of lipid absorbance and digestion is involved awaits further research.

In conclusion, fat/oil gavaged either at the beginning of or halfway through the active phases led to higher postprandial serum TG levels compared to that in passive phases. It appears that postprandial serum TG levels exhibit time of dependence upon single fat/oil applications under conditions which excluded shifting effect of altered food availability. This approach might provide a tool for better understanding how intestinal tissues respond to single fat/oil administration at various ZTs without disturbing their normal daily oscillations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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