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Original Article

The effect of supplementation of Isomaltooligosaccharide and emulsified medium-chain triglycerides on blood glucose, β-hydroxybutyrate and calorie intake in male Wistar rats

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SUMMARY

Background: A high concentration of energy substrate in circulation will suppress appetite and subsequent food intake. Medium-chain triglycerides (MCT) is a natural exogenous ketone source that can act as an energy substrate, increase blood ketone and suppress appetite. MCT emulsification increases its ketogenic properties. Interestingly, Isomaltooligosaccharide (IMO), a form of dietary fiber, can absorb and retain water, induce the stomach wall distention, and therefore could induce the afferent signal that causes appetite suppression. Despite those facts, there are no data available on whether a food supplement contains a combination of IMO and emulsified MCT (FC-MCT) could induce more appetite suppression.

Objectives: This research investigated the effect of FC-MCT on blood glucose, β-hydroxybutyrate (βHB), and calorie intake in male Wistar rats.

Methods: A total of 31 male Wistar rats were divided into four groups. 1) The MC group (n = 8) - MCT 10 ml/kg of body weight; 2) the FC group (n = 8) - combination of IMO and emulsified coconut oil or FC-CO 100% (w/v) 15 ml/kg of body weight; 3) the FM group (n = 7) - FC-MCT 100% (w/v) 15 ml/kg of body weight; and 4) the SU group (n = 8) - sucrose solution 20% (w/v) 15 ml/kg of body

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weight. Emulsified coconut oil acted as an isocaloric adjustor for IMO to balance MCT. Blood glucose and βHB were observed for 4 h, with 1 (one) hour measurement interval, presented as the area under the curve (AUC). The food intake was measured 4 h post supplementation.

Results: Compared with other groups, we observed low Blood glucose AUC ($p = 0.000$), total calorie ($p = 0.000$) and food intake ($p = 0.000$) and high βHB AUC ($p = 0.000$) in the FM groups.

Conclusion: We could successfully demonstrate that FC-MCT supplementation before a meal could suppress appetite and calorie intake. The caloric suppression seems to be mediated through the increased blood βHB concentration.

1. Introduction

In 2011, 1.46 billion people distributed in 199 countries worldwide were overweight. More than a half-billion among them are obese [1]. WHO reported that morbid obesity increases the mortality risk up to twelve times in people 25–35 years old. In children, the mortality risk caused by obesity is doubled [1]. Obesity has been predicted to cause 3.4 million deaths annually, 3.9% a year of life lost, and 3.8% disability-adjusted life year (DALY) [2]. Obesity causes death indirectly by increasing the risk to suffer from chronic diseases, such as type 2 diabetes mellitus, cardiovascular diseases, systemic hypertension, stroke, and cancer. All of those chronic diseases shorten the life expectancy of obese people by 6–7 years [1,3].

Obesity is directly associated with high caloric intake and low physical activity. Higher food intake is stimulated by appetite. Appetite is regulated physiologically by several mechanisms that aim to maintain body weight within a normal range. Those controls of food intake include glucostatic mechanism, aminostatic mechanism, lipostatic mechanism, energostatic mechanism, gastrointestinal (GI) tract wall distention, intestinal endocrine signaling mechanism, and neural response to different macronutrients [4–7].

Glucostatic mechanism refers to the regulation of calorie intake by the hypothalamus through the concentration of blood glucose. In aminostatic mechanism, amino acid concentration plays a more important role in hunger sensation. Based on this hypothesis, those who eat less protein in their diet tend to eat more until the amino acid concentration in their blood reaches enough level to suppress the hunger centre in the brain [5,6]. Lipostatic mechanism involves leptin signaling, a hormone produced by the adipocytes as a response to fat accumulation. In adipose tissue, when fat storage is abundant, leptin production increases. Leptin will suppress the hunger centre in the hypothalamus [8]. The food itself can affect the cells lining the GI walls to produce several types of orexigenic hormones which can induce appetite such as Ghrelin, and anorexigenic hormones which can suppress appetite such as Glucagon-like Peptide-1 (GLP-1) and Cholecystokinine (CCK) [5,8]. Stomach wall distention exerted by the existence of food can also induce afferent signals to the hypothalamic hunger centre to suppress food intake. Both of these mechanisms act to suppress food intake directly because they depend on the existence of food material within the GI tract [9].

Our caloric intake is tightly coupled with the cellular energy need. When the cellular energy need increases, our body will send signals to induce the hypothalamic hunger centre and increase food intake. For example, hepatic energy state is associated with its oxidation–phosphorylation activity within its cells. When the oxidation–phosphorylation activity is high, hepatic cells will transmit afferent signals through the vagal nerve to the hypothalamic satiety centre in the brain, specifically in Ventromedial Hypothalamus (VMH) area to suppress food intake. The higher the food or calorie intake, the higher the oxidation activity within hepatic cells, and the lower the appetite. This calorie intake

regulation is called the energostatic mechanism [4]. The energostatic mechanism depends on the availability of oxidizable fuels in terms of caloric values regardless of their chemical structure.

Ketones and fatty acids are oxidizable fuels that contain more caloric values than glucose and amino acids so that, from the energostatic hypothesis perspective, can stimulate the satiety centre in VMH better than glucose and amino acids. There are three types of ketones produced by metabolism, β-hydroxybutyrate (βHB), acetoacetate, and acetone. Because the ratio between blood βHB and acetoacetate concentration is 3:1, βHB contributes more to energy metabolism than acetoacetate [10]. Therefore, in this research we emphasized blood βHB concentration observation as a representative parameter for blood ketone detection. In addition to their function as alternative fuels [11], ketones can function as a signal molecule that suppresses food or calorie intake [12], regulate gene expression [12,13], suppress inflammation [12,13], improve cardiac helath [14], and particularly has neuroprotective properties [14]. Medium-chain triglycerides (MCT) could act as a natural ketone supplement because it can be absorbed easily and directly through the intestinal wall and metabolized by the liver to produce ketones [15]. Several studies indicated that MCT supplementation could suppress appetite, improve lipid profile, and increase energy expenditure (EE) [16]. Its effect on appetite suppression seems to be mediated by the ketone concentration in blood circulation and also its direct metabolic effects [15,17,18]. Courchesne-Loywe et al. (2017) showed that consumption of emulsified MCT could induce ketogenic properties by increasing blood ketone concentration with an additional advantage such as low adverse effect compared to non-emulsified MCT [19].

In addition to MCT, dietary fibre can induce satiety and decrease calorie intake. It will stay longer in the stomach and its ability to bind water molecules can increase food material volume in the stomach and distends the stomach wall [20,21]. The stomach wall distention stimulates the gastric stretch receptors cells to transmit a signal to suppress food intake. Isomaltooligosaccharide (IMO) is one of the dietary fibres which has a low digestibility index [22]. It could also improve the lipid profile, faecal flora, and bowel function [23]. Despite those facts, there are no data available whether food supplement contains the combination of dietary fibre in the form of IMO and emulsified MCT (FC-MCT) could induce more appetite suppression.

In this experiment, rats were administered with FC-MCT orally. This compound should have a synergistic effect on rat calorie intake suppression. In this manuscript, we could successfully demonstrate that FC-MCT supplementation before a meal could suppress appetite and calorie intake. In the future, FC-MCT could be used as a food supplement which might have a tremendous impact on reducing the weight of obese patients and improving their metabolic profile.

2. Materials and methods

2.1. Research design

This research was conducted by randomized pre-test post-test control group design. Three to four months old male Wistar rats (*Rattus novergicus*) weighted 80–150 g, were acclimatized with standard chow diet AIN-93 M *ad libitum* [24] (Supplementary Table 1) for 14 days before the experiment. Their body weight was measured and recorded weekly. Rats which show decreasing body weight would be excluded from the study, because decreasing body weight is the sign of chronic stress, and it will confound the effect of supplement in suppressing food intake [25]. Rats were put into a separate cage, with 12 h of light–and–dark cycles. This research was conducted in animal laboratory in Widya Mandala Catholic University Surabaya. This research was approved by the Health Research Ethics Committee of Widya Mandala Catholic University Surabaya (No. 0723/WM12/Q/2018).

2.2. Supplement preparation: MC, SC, FC, and FM

In the control supplementation group, the MC and SC group, Medium Chain Triglycerides Oil (MCT), and sucrose solution were administered orally. MCT was purchased from PT. Okusi Biotech Asia,

Jakarta, Indonesia. Sucrose 20% was prepared from 20 g of refined sugar available on the local market (Gulaku, produced by PT. Sugar Group Company, Jakarta, Indonesia) diluted in 100 ml of distilled water. Sucrose solution was chosen as a control because it could suppress endogenous ketones or βHB production and its effect in increasing blood glucose will suppress food intake in rats. The food supplementation in one of the experimental groups, the FC group, was a combination of isomaltooligosaccharides (IMO) and emulsified coconut oil (described as 100% FC-CO w/v) or marketed as Fibercreme™, were purchased from PT. Lautan Natural Krimerindo, Mojokerto, Indonesia. Emulsified coconut oil acted as an isocaloric adjustor for IMO to balance MCT. In another experimental group, the FM group, the combination of IMO and emulsified MCT (described as 100% FC-MCT w/v, purchased from PT. Lautan Natural Krimerindo) were administered orally. Emulsified MCT was produced by mixing MCT with caseinate, distilled mono- and diglycerides as emulsifiers, and dipotassium phosphates and sodium polyphosphates as stabilizers. Silica dioxide was added as an anti-caking agent.

2.3. The oral intragastric gavage treatment of rats with different supplements

Rats were divided equally into four groups according to the supplementation used as described above: two experimental groups (FC and FM) and two control groups (MC and SU). Each group consists of 8 rats [14]. Before experimental setups, rats were put into fast overnight for 10–12 h. The Supplement was administered orally once through intragastric gavage using a syringe with specific doses. MC groups received 10 ml/kg body weight MCT solution. All other groups received each 15 ml/kg body weight of FC-CO solution (for FC-group), FC-MCT solution (for FM-group), and sucrose solution 20% as described above (for SU-group). The amount of MCT in the MC group was based on the previous study design [15]. The dose of FC-CO, FC-MT, and 20% sucrose solution was calculated based on the same isocaloric amount compared with MCT receiving groups. The detailed composition and comparison of each experimental supplement are listed in Supplementary Table 2.

2.4. Blood sample preparation, blood glucose- and βHB concentration measurement, and food intake measurement

The animals were fasted for 10–12 h *ad libitum* before blood glucose and βHB measurement. An intravenous cannula was inserted into the tail dorsal vein to provide access to repeated glucose and βHB measurement. During the process, rats were placed into restrainers. Peripheral vein whole blood samples were taken for analysis of glucose and β-hydroxybutyrate (βHB) concentration using commercially available FreeStyle Optium glucose and βHB monitoring system (Abbot Laboratories, Chicago, Illinois, USA). Blood glucose and βHB were measured at 0, 1, 2, and 4 h (h) after the test substance administration. The first measurement at 0 representing the fasting blood glucose and βHB concentration. Blood glucose and βHB measurement unit at 0, 1, 2, and 4 h for each rat was described as the area under the curve (AUC). The area under the curve (AUC) reflects the actual body exposure to certain substances after their administration. It depends on the rate of substance elimination from the body and its dose of administration. AUC calculation (symbolized with A) was carried out using a linear trapezoidal method (see Supplementary Fig. 1) [26]. The general formula for the trapezoid is as follows,

$$A_{\text{Trapezoid}} = \frac{1}{2} \cdot (a + c) \cdot h \tag{1}$$

2.5. Food intake measurement

For food intake measurement in rats, After the first measurement of blood glucose and βHB concentration at $t = 0$, rats were given access to standard food (AIN-93M) as much as 10 g of food per rat. At the end of the experiment ($t = 4$ h), the remaining food was weighed and documented.

Table 1
Rat body weight across the experiment groups.

Group	Bodyweight ± SD (gram)
MC	188.38 ± 16.48
FC	199.38 ± 26.60
FM	187.14 ± 21.65
SU	206.38 ± 21.40

2.6. Statistical analysis

The data were analyzed statistically with SPSS v17.0 (IBM company, New York, USA) using Shapiro–Wilk Kruskal–Wallis and One-way ANOVA methods presented graphically as the mean ± standard deviation (SD) with Graphpad Prism™ 5.0 (San Diego, USA). All results were interpreted as significant if $p < 0.05$. The data were tested for normality spreads, using Shapiro–Wilk methods. It is considered "normal" if $p > 0.05$. All of the data would be tested for homogeneity using Lavene's test before *post-hoc* testing. All the mean comparisons were carried out using the Bonferroni post-hoc test if the data variance were homogenous and using the Tamhane test if the data variance were not homogenous. Correlation between blood glucose AUC, βHB AUC and total caloric intake would be analyzed using Pearson's test when their linearity test was insignificant ($p > 0.05$).

3. Results

3.1. Bodyweight, fasting blood glucose and βHB concentration

The body and baseline blood glucose and βHB concentration across the group are listed in Tables 1 and 2. There was no significant difference between groups in terms of body weight, fasting blood glucose, and βHB concentration ($p > 0.05$).

3.2. The supplementation with combination of IMO and emulsified MCT (FC-MCT) indicated as FM-groups decreases rat blood glucose concentration in certain measurement points

As mentioned above, blood glucose concentration was measured at 1, 2, and 4 h after supplementation. The mean blood glucose (mg/dL) for each group is presented in Fig. 1. The results indicated that at 1h, blood glucose concentration in MC, FC, and FM groups is significantly lower than the SU group ($p < 0.05$). MC group is not significantly different from FM ($p = 0.876$) at 1h. MC group is also not significantly different from the FC group ($p = 0.902$) at 1h, whereas the FM group is significantly lower than the SU group at 1h ($p = 0.000$). At 2h, the FM group is the lowest compared to the three other groups ($p = 0.000$). There was no significant difference between MC, FC, and SU groups at 2h. At 4h, blood glucose in the FM group is lower than in the SU group ($p = 0.006$), although it was not significantly lower compared to MC and FC groups.

3.3. The supplementation with combination of IMO and emulsified MCT (FC-MCT) indicated as FM-groups increase rat blood βHB concentration in certain measurement points

Blood βHB concentration was measured at 1, 2, and 4 h after supplementation. The measurement data for each group is presented in Fig. 2. At 1, 2, and 4h βHB concentration in MC and FM group was higher compared to other groups ($p = 0.000$). At 4 h, βHB concentration in the FM group was higher than the MC group ($p = 0.028$).

Table 2
Blood glucose and βHB concentration in rats at baseline.

Group	Fasting blood glucose ± SD (mg/dL)	Fasting blood βHB ± SD (mmol/L)
MC	92.25 ± 23.57	2.15 ± 0.57
FC	91.25 ± 13.77	1.90 ± 0.52
FM	96.00 ± 13.13	1.51 ± 0.41
SU	88.25 ± 7.98	2.00 ± 0.34

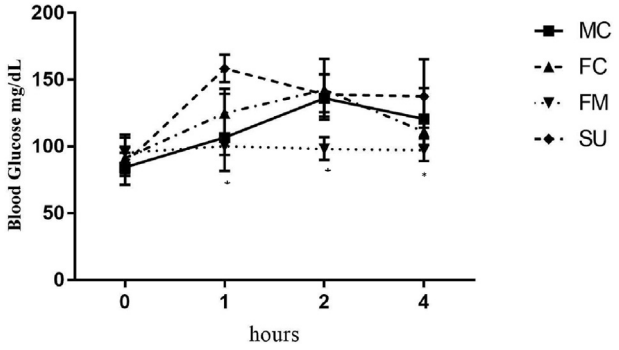

Fig. 1. Blood glucose concentration in rats at 1,2 and 4 h after supplementation. The asterisk indicates a significant difference compared to other groups ($p < 0.05$).

3.4. The supplementation with combination of IMO and emulsified MCT (FC-MCT) decreases rat blood glucose and increases rat βHB concentration, indicated by AUC parameters

The results of blood glucose and βHB concentration AUC for each group are presented in Figs. 3 and 4, respectively. Fasting glucose and βHB were used as baseline values in AUC calculation. Blood glucose AUC in FM group is the lowest compared to other groups ($p = 0.000$). Blood glucose AUC in the FM group is significantly lower than the FC group even though the FC-CO solution contains the same carbohydrate content as the FC-MCT solution. Low blood glucose AUC in the FM group can be attributed to low food intake in that group. Pearson test shows that blood glucose AUC is strongly correlated with

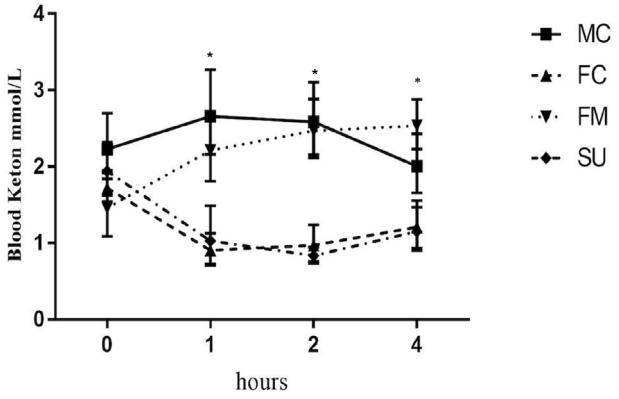

Fig. 2. Blood βHB concentration for every rat groups at 1, 2, and 4h after supplementation. At 1, 2, and 4h βHB concentration in MC and FM group were higher compared to three other groups. At 4 h, βHB concentration in the FM group was higher than the MC group. The asterisk indicates a significant difference compared to other groups ($p < 0.05$).

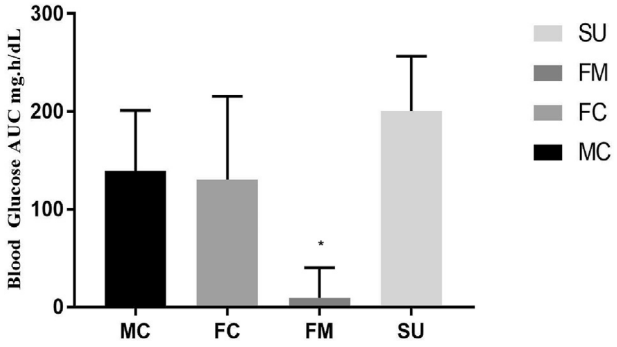

Fig. 3. Blood Glucose concentration AUC comparison between groups. Blood glucose AUC in The FM group is significantly the lowest compared to other groups. An asterisk indicates a significant difference compared to the other groups ($p < 0.001$).

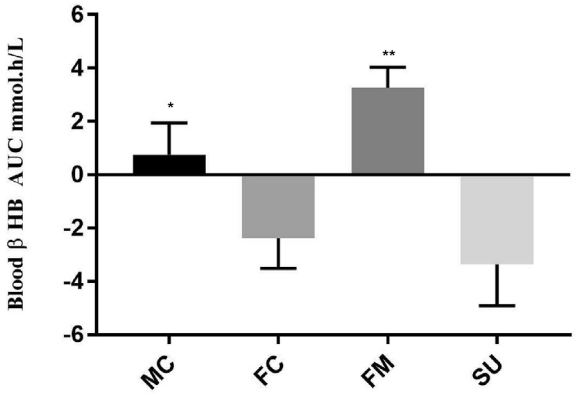

Fig. 4. Blood βHB concentration AUC comparison between groups. Blood βHB AUC are inversely correlated with blood glucose AUC. Blood βHB AUC in the FM group is significantly the highest compared to other groups. An asterisk (*) indicates a significant difference compared to other groups ($p < 0.05$) (**) indicates a significant difference with (*) ($p < 0.05$).

food intake ($r = 0.72$ and $p = 0.000$). Meanwhile, blood glucose AUC is inversely correlated with blood βHB AUC ($r = -0.65$ and $p = 0.000$). Based on the facts that 1 mol of glucose could provide 10 ATP, 1 mol of βHB could provide 13 ATP and 1 mol of ATP equals 7.3 Kcal of energy [33], then the total potential energy or oxidizable fuel available in blood circulation for each group during 4 h are presented in Fig. 5. There is no significant difference between groups in terms of the availability of total energy AUC ($p = 0.169$).

3.5. The supplementation with combination of IMO and emulsified MCT (FC-MCT) decreases rat appetite (food intake) and caloric intake

In this experiment, Rat appetite (quantified as food intake) was measured by subtracting its mass in the beginning with the remaining food at 4h. It was measured using a digital scale. The results are listed in Table 3. To eliminate the effect of body weight on food intake and normalize its distribution, the food intake data were divided by body weight.

FM group has the lowest food intake compared to the three other groups ($p = 0.000$, indicated by "*" in Table 3). Despite FC and MC group ate less food compared to the SU group ($p = 0.001$ and $p = 0.037$), the FM group ate less food than FC and MC group ($p = 0.010$ and $p = 0.000$). If the data listed in Table 3 are corrected by converting it into total calorie food consumed added with calories contained

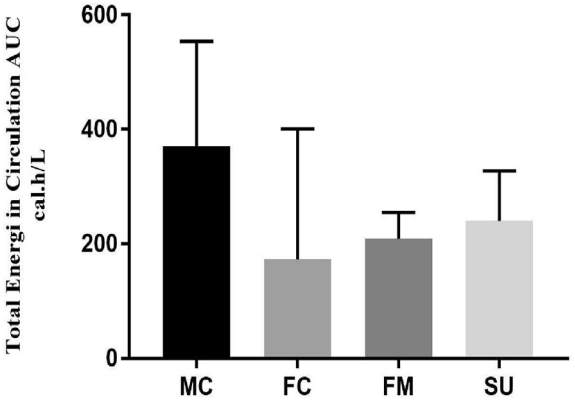

Fig. 5. The total energy in circulation AUC comparison between groups. Even though the mean availability of total energy in the MC group is the highest, statistically there is a significant difference between all groups.

Table 3
Food intake measured at 4h.

Group	Food intake ± SD (gram)	Food intake corrected by body weight ± SD (gram)
MC	5.38 ± 1.69	0.029 ± 0.008
FC	4.50 ± 2.07	0.023 ± 0.009
FM	1.29 ± 1.28*	0.007 ± 0.007*
SU	8.38 ± 0.92	0.041 ± 0.009

in their supplement, this is no significant differences between MC, FC, and SU group, but there is still a significant difference between FM groups compared to three other groups ($p = 0.000$). Those data are presented in Fig. 6.

4. Discussion

FC-MCT supplementation before a meal could suppress appetite and calorie intake. This hypothesis has been proved by significant differences in food intake and total caloric intake between groups of

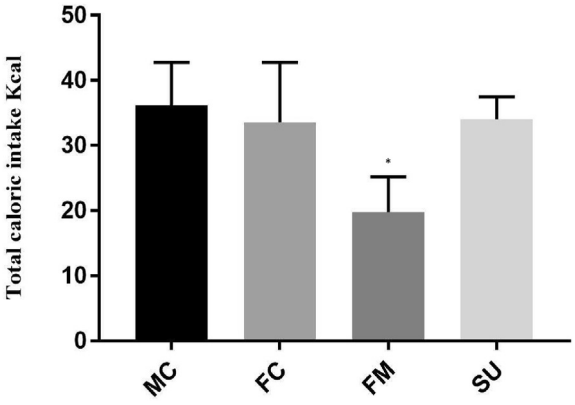

Fig. 6. Total caloric intake comparison between groups. No significant difference was observed between MC, FC, and SU groups. Total caloric intake in the FM group is significantly lower compared to the three other groups. An asterisk indicates a significant difference compared to the other groups ($p < 0.001$).

rats. Indeed, our data showed that total caloric intake in the FM group is the lowest compared to the three other groups. Nevertheless, the difference in food intake (Table 3) and total caloric intake (Fig. 5) cannot be attributed to the difference in the availability of oxidizable fuels in the blood circulation which is represented by the value of total energy AUC ($p = 0.169$, see Fig. 6). The availability of oxidizable fuels in FM groups is not significantly higher compared to the other three groups, although this group consumed significantly less food compared to the other three groups ($p = 0.000$).

Based on our results in Figs. 5 and 6, we postulated that appetite did not only depend on the total caloric intake but also depends on the nature of the energy substrates. Those energy substrates did not function only as fuel but also as signal molecules with various effects, such as appetite suppression which works on hypothalamic neurons and maintain cholecystokinin (CCK) hormone in blood circulation as an anorexigenic hormone [27]. Ketones also increase the expression of antioxidant defense genes, affect gene expressions regulations through its effect in inhibiting histone deacetylase (HDAC) enzyme and modulates senescence [28].

The similarity of total energy AUC between groups showed us that cellular metabolism can utilize MCT as alternative energy to substitute glucose and as an appetite suppressant. Appetite controls the availability of energy substrate within circulation until it reaches a certain point, then rats will stop eating. This phenomenon supports the energostatic mechanism of appetite regulation hypothesis which explains that organisms' ingestive behavior is affected by the concentration of oxidizable fuels that is accessible to body cells regardless of their difference in chemical structures, whether they are derivates of carbohydrate, lipid, or proteins [4,6,7]. Additionally, low caloric intake in the FM group despite no significant difference in the concentration of oxidizable fuels compared to other groups indicated the involvement of another factor in regulating appetite besides energostatic mechanism, most likely the blood βHB.

In this manuscript, we demonstrated that rat blood glucose AUC in the FM group is the lowest ($p = 0.000$) among other groups (Fig. 3), whereas rat βHB concentration AUC (Fig. 4) is the highest ($p = 0.000$). These data are in accordance with previous findings that MCT supplementation can suppress blood glucose concentration and increase blood βHB concentration in rats [15]. Other studies also concluded that appetite suppression was mediated by the increase of blood ketone concentration [15,17,18]. Blood ketone does not only play a role as an energy alternative to glucose, but it also acts as a signal molecule that can suppress the production of *ghrelin*, an orexigenic hormone [29]. Ketone, in the form of βHB, is suggested to suppresses appetite by directly affecting certain neurons in the central nervous system, although the identity of those neurons is still unknown [12]. The higher the ketone concentration, the more abundant the signal molecules which can suppress the appetite. Therefore, the blood βHB parameter is one of the important markers to measure the suppression of appetite.

High blood βHB concentration in FM groups observed in this manuscript indicates that emulsified MCT in FM-MCT formula is more ketogenic compared to non-emulsified MCT even though non-emulsified MCT contains more than 3-folds higher MCT than FM-MCT formula (see Supplementary Table 2). This statement is in accordance with previously reported studies that MCT emulsification increases its ketogenic effect because it can be digested easier by lipase enzyme and absorbed faster than its non-emulsified counterpart [19]. Additionally, the effect of FC-MCT supplementation on blood βHB concentration occurred indirectly through its effect on suppressing food intake. Low food intake increases blood βHB concentration because it directs the body to break down its energy storage in the form of fat to yield fatty acid in the blood circulation and subsequently metabolized by the liver to produce ketones. Regardless of the blood ketone concentration, the increase of fatty acid accumulation and its subsequent oxidation in the liver *per se* is enough to induce afferent signals transmitted through the vagal nerve to suppress the hunger center in the lateral hypothalamic. This signaling circuitry, from adipose tissue, liver, and brain, is called the liver-brain-adipose tissue axis [4,30].

Interestingly, MCT which contains medium-chain fatty acids (MCFA) can go through the inner membrane of mitochondria without L-carnitine as shuttle and carnitine palmitoyltransferase-I (CPT-I) enzyme as its catalyst, because MCFA is oxidized easier and faster than long-chain fatty acids (LCFA). Previous studies concluded that the increase of lipid oxidation by hepatocytes and enterocytes can suppress appetite regardless of blood ketone concentration [5,31]. Based on that mechanism, MCFA has more satiating properties compared to LCFA which is commonly found in various food products such as margarine, palm oil, and coconut oil [32]. In fact, Coconut oil (FC—CO groups) contains only 5% MCFA

[33], which explains why emulsified MCT in the FC-MCT formula can suppress appetite better than FC-CO (see Fig. 7).

High food intake in the SU group could indicate the effect of fructose in increasing food intake. In the GI tract, one mole of sucrose can be hydrolyzed into one mole of glucose and fructose. Glucose in the GI tract contributes directly to blood glucose and supplies energy for various body cells, whereas fructose is converted into fatty acids and increases very-low-density lipoprotein (VLDL) concentration in plasma [34]. Fructose increases food intake in rats by several mechanisms. It increases appetite directly by stimulating the hypothalamic hunger centre and indirectly by increasing the ghrelin-hormone production [34,35]. Additionally, previous studies indicated that glucose has the same properties as fructose in increasing caloric intake and body weight [36]. Those studies supported the hypothesis that ingestive behaviour does not only depend on total caloric intake and the concentration of oxidizable fuel in blood but also on the chemical nature of fuel substrates because they act as signal molecules.

Statistical analysis of appetite suppression through the βHB concentration could partially explain the effect of FC-MCT, because of their moderate strength correlation coefficient ($r = -0.437$ and $p = 0.014$). Even though the non-emulsified MCT (see Fig. 6) can increase blood βHB concentration significantly compared to two other groups (FC and SU); it could not suppress total caloric intake in the MC group. This result indicates that MCT supplementation in the MC group could not increase blood βHB concentration high enough to suppress hypothalamic hunger center. Additionally, IMO content in the FC group provides an extra effect in suppressing total caloric intake. IMO, which acted as dietary fiber with low digestibility and glycemic index (Glycemic Index = 34 [37]), has a synergistic effect with MCT in reducing caloric intake. IMO can stay longer in the GI tract, decrease glucose absorption, and retain water so it follows that it can distend the gastric wall and induce mechanoreceptors in the gastric wall to transmit an afferent signal to suppress food intake. Reduced food intake would decrease blood glucose concentration. Blood glucose concentration inversely correlates s with blood βHB concentration ($r = -0.653$ and $p = 0.000$). The higher the ketone concentration, the lower the glucose concentration. Theoretically, low glucose concentration is caused by reduced food intake, meanwhile, ingestive behavior which controls food intake is regulated by the blood ketones concentration.

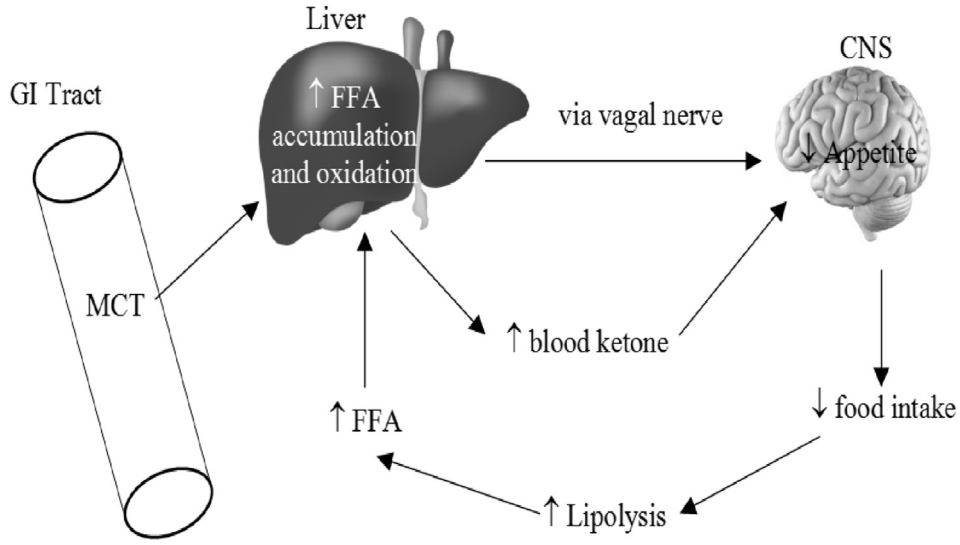

Fig. 7. The mechanism of caloric intake suppression exerted by Medium Chain Triglycerides (MCT) supplementation. MCT consumed is digested and distributed through Gastrointestinal (GI) tracts. Accumulation of MCT in the liver could lead to accumulation and oxidation of Free Fatty Acids (FFA) and increases in blood ketone concentration, which generate signal via the vagal nerve in the central nervous systems (CNS) to suppress appetite. The decrease of appetite could suppress the food intake, which may result in higher lipolysis, accumulation of free fatty acids in the body, and may trigger weight loss.

Whether FC-MCT supplementation can reduce rat body weight is still unclear. However, the fact that FC-MCT supplementation can suppress total caloric intake, long-term administration of FC-MCT before meals should be able to reduce body weight, and also might improve other metabolic profiles such as lipid profile, HbA1C concentration, fasting insulin concentration, or uric acid concentration. Further studies are needed to evaluate those parameters.

5. Conclusions

In this research, we observed that FC-MCT suppresses total caloric intake in male Wistar rats. Caloric intake suppression is likely mediated by the increased blood ketones concentration. The higher the blood ketone concentration, the lower the total caloric intake. MCT emulsification in FM-MCT formula has more ketogenic effects compared to non-emulsified MCT. Low blood glucose concentration in the group given FM-MCT occurred because they had low food intake. Taken together, we could successfully demonstrate that FC-MCT supplementation before a meal could suppress appetite and subsequent calorie intake. Additionally, this research concludes that ingestive behavior did not only depend on the total available oxidizable fuel in circulation, in the form of ketones and/or glucose but also depends directly on ketones as signal molecules within blood circulation.

Declaration of competing interest

The author declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nutos.2021.03.007.

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