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Research article

Effects of crocodile (*Crocodylus siamensis*) oil and palm oil on leptin-activated signal transducer and activator of transcription 3 signaling pathway and hepatic fat accumulation in rats

Pitchaya Santativongchai[a], Krittika Srisuksai[b], Kongphop Parunyakul[b], Paweena Aendo[c], Urai Pongchairerk[d], Prapassorn Boonsoongnern[d], Wirasak Fungfuang[b], Phitsanu Tulayakul[c,*]

[a] *Bio-Veterinary Sciences (International Program), Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand*

[b] *Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

[c] *Department of Veterinary Public Health, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*

[d] *Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand*

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Abstract

Importance of the work: Although fat is an essential nutrient, its accumulation is associated with chronic diseases in animals and humans. Different types of oil differently alter food intake and energy expenditure through regulation of leptin signaling.

Objectives: To investigate the effect of palm oil (PO) and crocodile oil (CO) consumption on leptin signaling in rats.

Materials & Methods: Male Wistar rats (7 rats/group) were divided into control, PO and CO groups. The rats were treated with the oils or water (control) supplied using oral gavage once daily (3 mL per kg body weight) for 7 wk.

Results: The CO group had significantly ($p < 0.01$) lower food intake than the control group. The calculated total area of fat droplets in the liver tissues of the CO group was significantly ($p < 0.01$) lower than for the PO group. The increased phosphorylation of the signal transducer and activator of transcription 3 accompanied by hunger suppression with the attenuation of Neuropeptide Y expression was higher in the CO group.

Main finding: CO lowered hunger expression and hepatic fat accumulation due to the increased promotion of leptin sensitivity compared to PO. Further study should investigate CO consumption in other animals for further alternative uses in medical therapy.

* Corresponding author.
E-mail address: fvetpnt@ku.ac.th (P. Tulayakul)

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Introduction

During the last decade, nutrition has become an important health concern, especially as fat is one of the essential nutrients that humans must consume in their diets (World Health Organization, 2020). There are different types of fats, including unsaturated fats, which are important for health when consumed in small amounts as part of a balanced diet (World Health Organization, 2020). However, diets containing high levels of animal fats and saturated fatty acids have been associated with an increased risk of cardiovascular diseases (Briggs et al., 2017; Willett, 2012), type 2 diabetes mellitus (T2DM), glucose tolerance, dyslipidemia and metabolic syndrome (Karaderi et al., 2015; Ruiz-Nunez et al., 2016). Nonetheless, fats are still one of the main macronutrients and components in common food products (Briggs et al., 2017).

Central pathways control the regulation of body weight and food intake by the fat-derived leptin hormone (Schwartz et al., 2000; Cone et al., 2001). Leptin acts on the brain to regulate hunger expression and neuroendocrine function, mainly via receptors in the hypothalamus (Munzberg et al., 2003). Pro-opiomelanocortin neurons in the arcuate nucleus (ARC), the paraventricular nucleus, the dorsomedial hypothalamic nucleus (DMH), the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic area are important targets of leptin and express the leptin receptor (ObRb) (Munzberg et al., 2003). Leptin signaling is phosphorylated on tyrosine residues located on the ObRb mediated by the activation of Janus-activated kinase 2 (JAK2) proteins (the intracellular sequences of ObRb) (Bjorbaek et al., 1997). Subsequently, cytoplasmic signal transducer and activator of transcription 3 (STAT3) proteins are bound to the phosphorylated Y1138 residue and tyrosine-phosphorylated by JAK2, then dimerized and translocated into the nucleus to regulate gene transcription resulting in a satiety response (Bjorbak et al., 2000; Munzberg et al., 2003). In the ARC, neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons coexpress leptin receptors and are negatively regulated by leptin. Consequently, the leptin signaling additionally decreases NPY, a stimulator of food intake and a regulator of lipid metabolic homeostasis (Jang et al., 2000; Oberto et al., 2003; Park et al, 2017b).

Palm oil (PO) is used primarily in food manufacturing, beauty products and cooking in many countries throughout the world because of the belief that plant oil is healthier than animal oil (Chong and Ng, 1991; Kosulwat, 2002). Although PO is likely to be less detrimental than animal oils, the high proportions of saturated fatty acids in the oil could also contribute to metabolic syndrome and cardiovascular diseases (Ueshima et al., 2008; Brouwer et al., 2015; Naphatthalung et al., 2018). Additionally, PO could negatively modulate glucose homeostasis, metabolic parameters and cytokine gene expression in the liver and adipose tissue (Miyamoto et al., 2020), which in total support the current concerns about the effects of PO on health impairment.

Crocodile fat is one of the by-products from the crocodile industry that is generally discarded. In addition, crocodile oil is one of the natural oils that has been reported to be very effective in the treatment of several ailments and is used as a component in cosmetics (Shim-prydon and Camacho-Barreto, 2007) resulting from its high levels of unsaturated fatty acids (Buthelezi et al., 2012; Li et al., 2017; Maroon and Bost, 2006; Santativongchai et al., 2020). Crocodile oil (CO) has been traditionally used worldwide (Kelly, 2006; Shim-prydon and Camacho-Barreto, 2007), such as for allergy and skin problems (Shim-prydon and Camacho-Barreto, 2007; Venter et al., 2016). In addition, the anti-inflammatory activity of CO has been reportedly suggested as being due its higher level of omega-3 fatty acids compared to a saturated fatty acid-rich diet including animal oils and PO (Buthelezi et al., 2012; Santativongchai et al., 2020; Sastravaha et al., 2016). However, there has been little investigation on leptin signaling and the fat-related health concerns of CO. Therefore, CO was included in the current study to investigate whether it could be an alternative to cooking oils.

The current study aimed to investigate whether PO and CO consumption led to different effects on leptin signaling by emphasizing hunger suppression and the histopathology of liver fat accumulation affected by the hormones in rats. The insight gained from the current study could be applied for further development of the use of CO in other animals and humans.

Materials and Methods

Ethics statements

Animal procedures were approved by the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand (Approval no. ACKU65-VET-090, 2022) according to the Guidelines for Animal Care and Use under the Ethical Review Board of the Office of National Research Council of Thailand (NRCT).

Crocodile oil extraction

Abdominal fat samples were collected from slaughtered Siamese crocodiles (*Crocodylus siamensis*) obtained from crocodile farms in Nakhon Pathom and Kanchanaburi provinces, Thailand. The crude fat samples were stored at -20°C until the extractions were performed.

CO was extracted using the wet, cold-pressing method according Santativongchai et al. (2020). Briefly, the samples were pressed through two layers of filter cloth using distilled water and a ratio of 1:1 (weight per volume). Subsequently, the solution was left until the mixture had separated, after which the clear oil was evaporated and stored at room temperature until used.

Oil supplementation in rats

Male Wistar rats (*Rattus norvegicus*) were obtained from Nomura Siam International Co., Ltd., M-CLEA Bioresource Co., Ltd., Bangkok, Thailand. Animals were housed in a temperature-controlled room (25°C ± 2°C) with 60–70% humidity and a 12 hr artificial light then dark cycle. The rats (n = 7/group) were randomly divided into three groups: control, PO and CO groups. The animals were fed *ad libitum* with a regular control diet until the end of the experiment. At age 24 wk (mean ± SD, 500.43 ± 40.85 g body weight), the PO and CO groups were supplemented with commercial PO and the extracted CO based on oral gavage once a day at 3 mL per kg body weight for 7 wk, whereas the control group received only water. Body weight, food intake and caloric intake were measured and calculated throughout the experiment, where the caloric intake per day was calculated as (calories of the regular diet (kcal/g) × food intake per day (g/day)) + calories of the water or oils (water, 0 kcal/day; PO, 13.5 kcal/day; CO, 12 kcal/day).

Sample collection

At the end of the experiment, rats were fasted overnight (10±2 hr) and euthanized using the intraperitoneal injection of 40 mg per kg pentobarbital sodium. Blood samples were collected and centrifuged at 2,200×g for 15 min at 4°C. The serum samples were stored at -20°C until analysis. Livers and abdominal fats were dissected and weighed. Liver samples were prepared for histological examinations. Brain samples were fixed in 10% neutral buffered formalin for further processing of paraffin sections.

Serum measurements

The serum concentrations of total cholesterol (CHOL), triacylglycerol (TAG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using a Hitachi 7080 analyzer (Hitachi; Japan). The serum levels of leptin hormone were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Cat no. RAB0335, Sigma-Aldrich®; USA; sensitivity, 30 pg/mL; detection range, 10.97–8,000 pg/mL).

Histological examinations

Excisional liver biopsies fixed in 10% neutral buffered formalin were used for histological examination. To determine the structure and lipid accumulation in the liver, deparaffinized sections (5 µm thickness) were processed for hematoxylin and eosin (H&E) staining (n = 3/rat). Then, the sections were observed for histopathological changes under a microscope.

Oil Red O staining was performed with frozen sections of liver samples in optimum cutting temperature compound. Briefly, cryostat-cut sections (7 µm, n = 3/rat) were immediately fixed in 10% neutral buffered formalin and washed with 60% isopropanol. Then, the sections were incubated in Oil red O working solution, counter-stained with hematoxylin and cover-slipped. The average area, perimeter and total area of fat droplets in the Oil Red O sections were analyzed using iSolution DT (IMT i-Solution Inc., USA).

Immunohistochemistry

Rat brain sections (n = 3/rat) were prepared originating from the beginning of the DMH area until the end of the area, which included the dorsomedial area of the VMH (Ladyman and Grattan, 2013).

After the brain sections had been deparaffinized with xylene and rehydrated through graded alcohols, the sections were treated with Tris- ethylenediaminetetraacetic acid at pH 9.0 and 95–100°C for 20 min for antigen retrieval. Then, the slides were immersed in 3% hydrogen peroxide in Tris-buffered saline (TBS) for quenching of the endogenous peroxide. Subsequently, tissue sections were incubated with 5% bovine serum albumin (BSA) in TBS for 30 min, followed by incubation with rabbit monoclonal anti-phospho (P)-STAT3 (dilution, 1:100 (volume per volume, v/v; Abcam; UK) antibody or anti-NPY (dilution, 1:1000 (v/v); Abcam, UK) antibody in 1% blocking solution (1% BSA/TBS) at 4°C and left overnight. All slides were washed thrice with 0.1% TBS-Tween 20 and then incubated with 1:1000 (v/v) of horseradish peroxidase-conjugated goat anti-rabbit antibodies (Abcam; UK) for 1 hr at room temperature. After washing, the sections were stained with 3,3'-diaminobenzidine substrate for peroxidase reaction. The images were observed under an Olympus VS120 Virtual slide microscope (Olympus Corporation; Japan). Brown color expressions in the ARC area of the brain sections were analyzed using iSolution DT (IMT i-Solution Inc.; USA) using the same position with equal sizes.

Statistical analysis

Body weight gain, food intake, caloric intake, lipid parameters and leptin concentrations were reported as mean

± SD values. The data were analyzed using one-way analysis of variance, followed by Bonferroni's post-hoc test using the GraphPad Prism software (version 5; GraphPad Software Inc.; USA). A value of $p < 0.05$ was considered a statistically significant difference.

Results

Effects of crocodile oil on body weight gain, food intake and caloric intake

At the end of the experiment, there were no significant differences in body weight, body weight gain or abdominal fat weight among the groups. However, food intake was significantly decreased ($p < 0.01$) in rats supplemented with CO compared to the control group (Table 1). Nevertheless, a significantly higher caloric intake ($p < 0.01$) by the CO group was observed compared to the control group (Table 1).

Effects of crocodile oil on lipid parameters and serum leptin hormone

As seen in Table 1, none of the serum levels for lipid parameters was altered by PO and CO supplementation. Similarly, no significant difference was found in serum leptin levels. However, the CO group displayed trends to decrease serum levels of CHOL and LDL-C (Table 1), compared to the levels in the control group, and an increase in serum leptin levels relative to the control group (Table 1).

Crocodile oil reduces the risk of fat accumulation in liver tissues

The hepatocyte morphology of the liver sections was visualized using H&E staining. The histological findings of the control group showed normal morphology of hepatocyte (Fig. 1A), while fat droplet accumulation appeared in the cells of the PO and CO groups. However, the size of fat droplets accumulated in the cytoplasm of the CO group (Fig. 1C) was smaller than in the PO group (Fig. 1B).

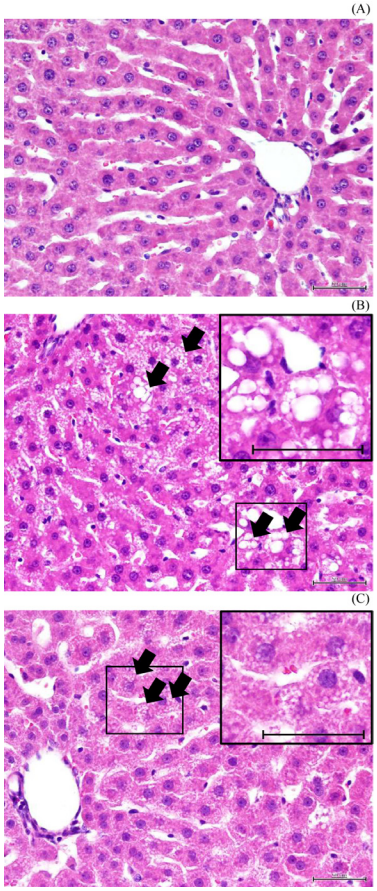

Fig. 1 Hematoxylin and eosin staining of liver tissue: (A) control group showing normal histological appearance of hepatocytes; (B) palm oil group showing microvesicular steatosis (arrows) with large-sized fat droplets in cytoplasm; (C) crocodile oil group showing microvesicular steatosis (arrows) with smaller-sized fat droplets than PO group, where scale bars = 50 µm and staining magnification = 400×

Table 1 Characteristics of rats (n = 7/group) fed control diet comprising water (control), palm oil (PO) and crocodile oil (CO) where caloric intakes already includes caloric density of oils (palm oil, 9 kcal/g; crocodile oil, 8 kcal/g)

Parameter	Control	PO	CO
Body weight (g)	516.29±49.10	516.57±50.77	523.86±16.61
Body weight gain (g)	1.16±2.979	1.13±4.458	1.66±3.973
Food intake (g/d/animal)	18.59±1.225^{a}	15.10±1.261^{b}	15.79±1.171^{c}
Caloric intake (kcal/d/animal)	63.72±4.199^{a}	62.95±4.127^{a}	66.13±4.016^{b}
Abdominal fat weight (g)	15.39±4.506	17.27±7.743	15.02±4.986
Total cholesterol (mg/dL)	73.13±19.01	66.10±16.46	63.82±8.980
Triacylglycerol (mg/dL)	138.30±60.28	83.68±12.87	90.40±33.84
LDL-cholesterol (mg/dL)	11.68±2.195	9.175±3.272	8.700±2.617
HDL-cholesterol (mg/dL)	53.58±18.57	46.40±14.32	46.70±7.643
Leptin (ng/mL)	4.040±3.281	4.303±3.532	5.403±2.300

Values (mean ± SD) in same row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Similarly, the Oil Red O stains showed a significantly lower amount of total area of fat droplets in the liver tissues of the CO group (Fig. 2C) than the PO group (Fig. 2B), although the control group (Fig. 2A) had a significantly lower total area compared to both (Fig. 2F). The average area per fat droplet in the PO group was significantly higher than in the control group; however, CO attenuated the effect to the same level as in the control group (Fig. 2D). Although no significant difference in the average area or perimeter per fat droplet (Fig. 2E) was observed between PO and CO groups, the amounts of these parameters were slightly lower in the CO group than in the PO group.

Phospho-signal transducer and activator of transcription and neuropeptide Y immunohistochemistry

The results showed visually apparent differences in hypothalamic P-STAT3 intensity between groups (Fig. 3). The analysis demonstrated that P-STAT3 immunoreactivity was significantly increased, mainly in the ARC of the CO group (Fig. 3D) compared to the control group (Figs. 3B), which showed less expressions in the ARC (Fig. 3H). In addition, P-STAT3 expression was evident in the VMH of the CO group (Fig. 3D). Conversely, the strong positive signal of NPY staining was significant in the ARC of the control group (Fig. 3E) compared to the PO (Fig. 3F) and CO groups (Fig. 3G), respectively (Fig. 3I). No immunohistochemical signal was found in the negative control sections (Fig. 3A).

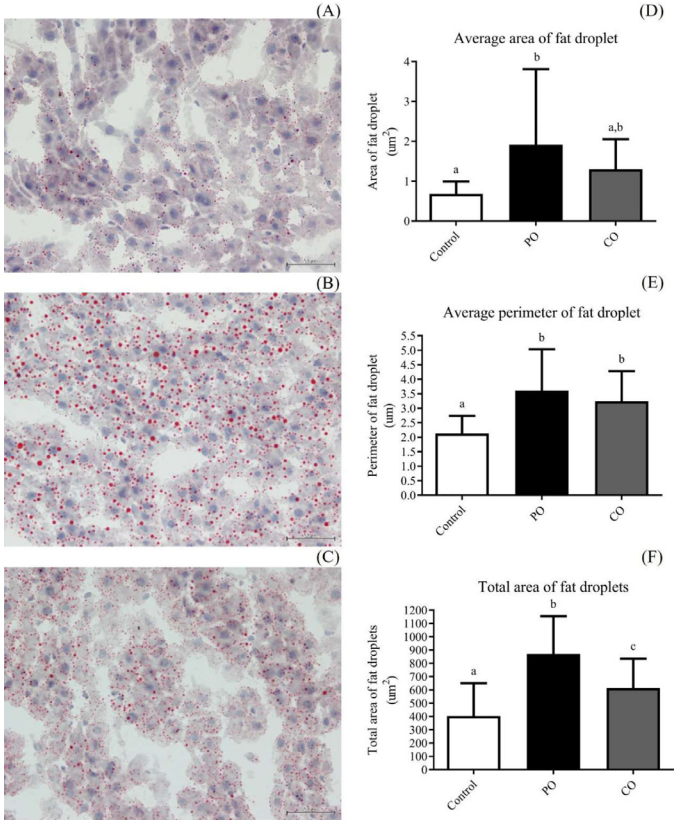

Fig. 2 Oil Red O stains of frozen liver sections counterstained with hematoxylin: (A) stains showing fat droplets (red spots) in control group; (B) palm oil group; (C) crocodile oil group, where scale bars = 50 µm, magnification = 400×. Bar graphs of liver fat droplets: (D) average area; (E) average perimeter; (F) total area, where values are mean ± SD (n = 7/group), different lowercase letters above columns in same graph indicate significant ($p < 0.05$) differences between groups.

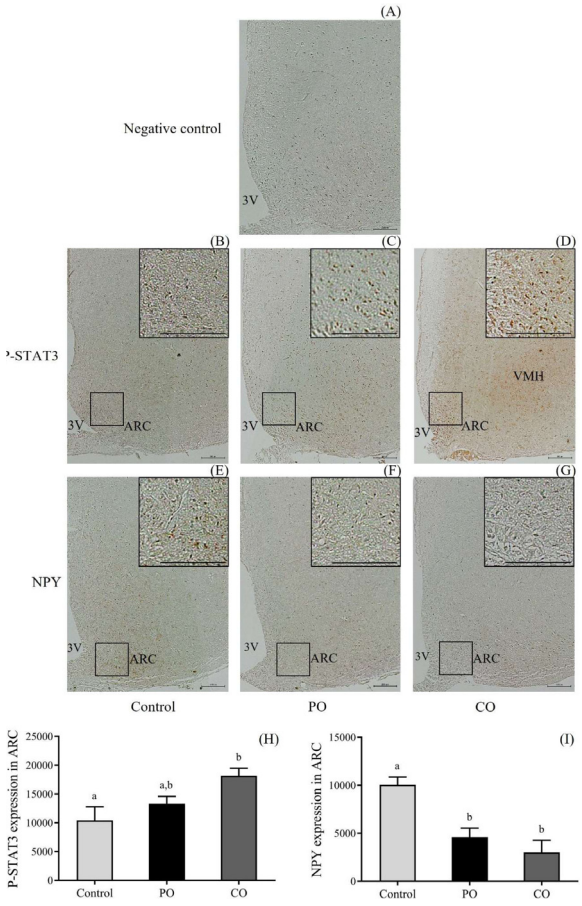

Fig. 3 Phosphoro-signal transducer and activator of transcription 3 (P-STAT3) and neuropeptide Y (NPY) immunohistochemical expression in rat hypothalamus, with magnified insets, where P-STAT3 expression increases mainly in nuclei of neurons in crocodile oil (CO) group (D) located in the arcuate nucleus (ARC) and also in ventromedial (VMH) hypothalamic nuclei compared to palm oil (PO) and control groups (C and B, respectively). NPY expression in hypothalamus decreases in CO group (G) and then the PO group (F), respectively, in medial parts of ARC compared to control group (E). Negative control (A) was investigated without primary antibodies included. Abbreviations: 3V = third ventricle, Scale bars = 200 µm, magnification = 100×, bar graphs indicate P-STAT3 (H) and NPY (I) expressions in ARC of each group reported as mean ± SD (n = 7/group), where different lowercase letters above columns in same graph indicate significant ($p < 0.05$) differences between groups.

Discussion

From the characteristics of the parameters in Table 1, CO group rats had significantly higher caloric intake than the control group, although food intake was found to be lower ($p < 0.01$). Furthermore, there was a trend of higher amounts of serum leptin hormone in the CO group compared to the other groups (Table 1). Other studies have stated that the fat-derived leptin hormone acts on the receptor in the hypothalamus to regulate appetite expression (Schwartz et al., 2000; Cone et al., 2001; Munzberg et al., 2003). Accordingly, the present study found that 3 mL per kg body weight of CO did not cause body weight gain in the rats when consumed for 7 wk, which might have been caused by the leptin-induced lower food intake, despite the higher caloric intake resulting from CO consumption. Furthermore, the results could suggest that this upregulated production of leptin might be an essential point that triggers higher leptin signaling, which led to hunger suppression when the rats received CO.

The lipid parameter results showed trends of lower serum CHOL and LDL-C in the CO group compared to the control group (Table 1). Other studies reported a high-fat diet typically led increased plasma concentrations of lipid parameters, such as CHOL, TAG and LDL-C (Lu et al., 2011; Martins et al., 2018). However, these changes could be prevented by fish oil or chia oil supplementation, or both, which could have been due to the increased beta-oxidation associated with the high levels of omega-3 fatty acids in the oils (Lombardo and Chicco, 2006; Hartweg et al., 2007; Martins et al., 2018; Fonte-Faria et al., 2019). Furthermore, other studies found that CO had higher levels of total omega-3 fatty acids (2.49 g per 100 g oil) compared to PO (0.30 g per 100 g oil) (Osthoff et al., 2010; Montoya et al., 2014; Santativongchai et al., 2020). Although there was no significant difference in the present study, this scope could be an interesting aspect for further studies associated with a lower serum lipid profile being affected by CO, which could lead to lower cardiovascular risk compared to a diet of saturated fatty acid-rich oil, including PO (Willett, 2012; Briggs et al., 2017).

The present study, demonstrated that leptin-related hunger suppression induced by CO, as assessed by the expressions of both P-STAT3 and NPY (Hubschle et al., 2001; Munzberg et al., 2003; Oberto et al., 2003), would be stimulated in higher levels compared to the control and PO groups. The results showed the increased phosphorylation of the JAK2-STAT3 signaling pathway accompanied by the attenuation of NPY in the CO group. Indeed, other studies suggested that a high-fat diet induced the activation of the hypothalamic JAK2-STAT3 signaling pathway resulting from leptin resistance in mice and rats (Augustine et al., 2006; Shapiro et al., 2011; Tsunekawa et al., 2017). However, the results in the present study showed that although there was increased activation of the JAK2-STAT3 signaling pathway in the PO and CO groups, the food intake of the rats was lower, in accordance with lower NPY expression than the control group. Thus, leptin resistance was not stimulated by CO consumption (Shewale et al., 2018). Additionally, high-fat diet consumption, such as a ketogenic diet, triggered a leptin-induced hypothalamic NPY reduction, resulting in a general reduction of perceived hunger and food intake (Scharrer, 1999; Paoli et al., 2015). In the present study, even though serum leptin levels were not significantly different, there was increased leptin sensitivity in the hypothalamus in the CO group, based on the P-STAT3 and NPY results. Consequently, it could be suggested that CO promoted leptin receptor sensitivity in the hypothalamus, which led to hunger suppression related to the food intake results.

The histological findings showed that most of the hepatocytes in the PO and CO groups exhibited microvesicular steatosis; however, the fat droplet size in the cytoplasm of the CO group was smaller than in the PO group, as shown in Figs. 1B and 1C, respectively. The Oil Red O staining technique showed that multiple fat droplets (red in color) were scattered throughout the cells of all groups (Figs. 2A–2C); however, the moderate numbers of minute fat droplets were clearly recognized in the control and CO groups, while the number of droplets in the PO group was higher. Several varieties of fat droplet sizes were contained within the cells of both PO and CO groups, indicating the microvesicular steatosis (and larger droplets) were more common in the PO group than the CO group (Figs. 2B and 2C, respectively). Microvesicular steatosis is a variant form of hepatic fat accumulation, which contains small fat vacuoles accumulating within hepatocytes. This histological condition indicates higher fat accumulation in the liver, which leads to other serious conditions, such as acute fatty liver (Hautekeete et al., 1990). Additionally, other studies have reported the critical role of leptin-related NPY reduction in the regulation of lipid metabolic homeostasis via the control of lipolysis and thermogenesis in a state of negative energy balance (Rojas et al., 2015; Park et al., 2017a, b). Certainly, fasting stimulates NPY, which increases food intake and body weight (Sahu et al., 1992), hence promoting adiposity when NPY is overexpressed (Zarjevski et al., 1993; Ruohonen et al., 2012). Conversely, the suppression of NPY signaling, affected by leptin, decreases body weight gain and adiposity in obese animals (Ishihara et al., 2006; Chao et al., 2011). Furthermore, leptin-associated NPY was suggested to play an important role in the fatty acid oxidation that occurred in response to caloric restriction-induced negative energy balance (Bruss et al., 2010). Consequently, it could be suggested that the decrease in liver fat accumulation might be related to the increase in fatty acid oxidation affected by higher leptin sensitivity in the hypothalamus when the rats received CO, which was in agreement with the present NPY immunohistochemistry results.

The present findings suggested that CO consumption could help reduce hunger expression and promote liver fatty acid oxidation in rats, which might be attributed to increased JAK2-STAT3 signaling as affected by the oil. Therefore, further study is required to investigate whether oral administration of CO could be used to promote leptin sensitivity (thus leading to lower hunger and hepatic fat accumulation) in other animals and humans to identify further alternative uses in medical therapy. However, further studies involving a lower serum lipid profile affected by CO should be performed to investigate other benefits—mainly attenuated cardiovascular risk qualification.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Augustine, R.A., Bunn, S.J., Grattan, D.R. 2006. Hypothalamic changes in phosphorylated STAT3 immunolabelling in a pseudopregnant rat model exhibiting central leptin resistance. Front. Neuroendocrinol. 27: 4–11. doi: 10.1016/j.yfrne.2006.03.009

Bjorbaek, C., Uotani, S., da Silva, B., Flier, J.S. 1997. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J. Biol. Chem. 272: 32686–32695. doi.org/10.1074/jbc.272.51.32686

Bjorbak, C., Lavery, H.J., Bates, S.H., Olson, R.K., Davis, S.M., Flier, J.S., Myers Jr, M.G. 2000. SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. J. Biol. Chem. 275: 40649–40657. doi.org/10.1074/jbc.M007577200

Briggs, M.A., Petersen, K.S., Kris-Etherton, P.M. 2017. Saturated fatty acids and cardiovascular disease: Replacements for saturated fat to reduce cardiovascular risk. Healthcare 5: e29. doi.org/10.3390/healthcare5020029

Brouwer, E.D., Watkins, D., Olson, Z., Goett, J., Nugent, R., Levin, C. 2015. Provider costs for prevention and treatment of cardiovascular and related conditions in low- and middle-income countries: A systematic review. BMC Public Health 15: 1183. doi.org/10.1186/s12889-015-2538-z

Bruss, M.D., Khambatta, C.F., Ruby, M.A., Aggarwal, I., Hellerstein, M.K. 2010. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. Am. J. Physiol. Endocrinol. Metab. 298: 108–116. doi.org/10.1152/ajpendo.00524.2009

Buthelezi, S., Southway, C., Govinden, U., Bodenein, J., du Toit, K. 2012. An investigation of the antimicrobial and anti-inflammatory activities of crocodile oil. J. Ethnopharmacol. 143: 325–330. doi.org/10.1016/j.jep.2012.06.040

Chao, P.T., Yang, L., Aja, S., Moran, T.H., Bi, S. 2011. Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab. 13: 573–583. doi.org/10.1016/j.cmet.2011.02.019

Chong, Y.H., Ng, T.K. 1991. Effects of palm oil on cardiovascular risk. Med. J. Malaysia 46: 41–50.

Cone, R.D., Cowley, M.A., Butler, A.A., Fan, W., Marks, D.L., Low, M.J. 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int. J. Obes. Relat. Metab. Disord. 25: S63–S67. doi.org/10.1038/sj.ijo.0801913

Fonte-Faria, T., Citelli, M., Atella, G.C., Raposo, H.F., Zago, L., de Souza, T., da Silva, S.V., Barja-Fidalgo, C. 2019. Chia oil supplementation changes body composition and activates insulin signaling cascade in skeletal muscle tissue of obese animals. Nutrition 58: 167–174. doi.org/10.1016/j.nut.2018.08.011

Hartweg, J., Farmer, A.J., Perera, R., Holman, R.R., Neil, H.A. 2007. Meta-analysis of the effects of n-3 polyunsaturated fatty acids on lipoproteins and other emerging lipid cardiovascular risk markers in patients with type 2 diabetes. Diabetologia 50: 1593–1602. doi.org/10.1007/s00125-007-0695-z

Hautekeete, M.L., Degott, C., Benhamou, J.P. 1990. Microvesicular steatosis of the liver. Acta. Clin. Belg. 45: 311–326. doi.org/10.1080/17843286.1990.11718105

Hubschle, T., Thom, E., Watson, A., Roth, J., Klaus, S., Meyerhof, W. 2001. Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. J. Neurosci. 21: 2413–2424. doi.org/10.1523/JNEUROSCI.21-07-02413.2001

Ishihara, A., Kanatani, A., Mashiko, S., et al. 2006. A neuropeptide Y Y5 antagonist selectively ameliorates body weight gain and associated parameters in diet□induced obese mice. Proc. Natl. Acad. Sci. U. S. A.103: 7154–7158. doi.org/10.1073/pnas.0510320103

Jang, M., Mistry, A., Swick, A.G., Romsos, D.R. 2000. Leptin rapidly inhibits hypothalamic neuropeptide Y secretion and stimulates corticotropin-releasing hormone secretion in adrenalectomized mice. J. Nutr. 130: 2813–2820. doi.org/10.1093/jn/130.11.2813

Karaderi, T., Drong, A.W., Lindgren, C.M. 2015. Insights into the genetic susceptibility to type 2 diabetes from genome-wide association studies of obesity-related traits. Curr. Diab. Rep.15: 83. doi.org/10.1007/s11892-015-0648-8

Kelly, L. 2006. Crocodile: Evolution's Greatest Survivor. Allen & Unwin. New South Wales, Australia.

Kosulwat, V. 2002. The nutrition and health transition in Thailand. Public Health Nutr. 5: 183–189.

Ladyman, S.R., Grattan, D.R. 2013. JAK-STAT and feeding. JAKSTAT 2: e23675. doi.org/10.4161/jkst.23675

Li, H.L., Deng, Y.T., Zhang, Z.R., et al. 2017. Evaluation of effectiveness in a novel wound healing ointment-crocodile oil burn ointment. Afr. J. Tradit. Complement. Altern. Med. 14: 62–72. doi.org/10.21010/ajtcam.v14i1.8

Lombardo, Y.B., Chicco, A.G. 2006. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. J. Nutr. Biochem. 17: 1–13. doi.org/10.1016/j.jnutbio.2005.08.002

Lu, J., Borthwick, F., Hassanali, Z., et al. 2011. Chronic dietary n-3 PUFA intervention improves dyslipidaemia and subsequent cardiovascular complications in the JCR: LA-*cp* rat model of the metabolic syndrome. Br. J. Nutr. 105: 1572–1582.

Maroon, J.C., Bost, J.W. 2006. ω-3 Fatty acids (fish oil) as an anti-inflammatory: An alternative to nonsteroidal anti-inflammatory drugs for discogenic pain. Surg. Neurol. 65: 326–331. doi.org/10.1016/j.surneu.2005.10.023

Martins, A.R., Crisma, A.R., Masi, L.N., et al. 2018. Attenuation of obesity and insulin resistance by fish oil supplementation is associated with improved skeletal muscle mitochondrial function in mice fed a high-fat diet. J. Nutr. Biochem. 55: 76–88. doi.org/10.1016/j.jnutbio.2017.11.012

Miyamoto, J.É., Reginato, A., Portovedo, M., et al. 2020. Interesterified palm oil impairs glucose homeostasis and induces deleterious effects in liver of Swiss mice. Metabolism 112: 154350. doi.org/10.1016/j.metabol.2020.154350

Montoya, C., Cochard, B., Flori, A., et al. 2014. Genetic architecture of palm oil fatty acid composition in cultivated oil palm (*Elaeis guineensis* Jacq.) compared to its wild relative *E. oleifera* (H.B.K) Cortés. PLoS One. 9: e95412. doi.org/10.1371/journal.pone.0095412

Munzberg, H., Huo, L., Nillni, E.A., Hollenberg, A.N., Bjorbiek, C. 2003. Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. Endocrinology 144: 2121–2131. doi.org/10.1210/en.2002-221037

Naphatthalung, J., Cing, L.S., Kanokwiroon, K., Radenahmad, N., Jansakul, C. 2018. Effects of six-week consumption of lard or palm oil on blood pressure and blood vessel H_2S in middle-aged male rats. Funct. Foods Health Dis. 8: 307–322. doi: 10.31989/ffhd.v8i6.521

Oberto, A., Mele, P., Zammaretti, F., Panzica, G., Eva, C. 2003. Evidence of altered neuropeptide Y content and neuropeptide Y_1 receptor gene expression in the hypothalamus of pregnant transgenic mice. Endocrinology 144: 4826–4830. doi.org/10.1210/en.2003-0197

Osthoff, G., Hugo, A., Bouwman, H., Buss, P., Govender, D., Joubert, C.C., Swarts, J.C. 2010. Comparison of the lipid properties of captive, healthy wild, and pansteatitis-affected wild Nile crocodiles (*Crocodylus niloticus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 155: 64–69. doi.org/10.1016/j.cbpa.2009.09.025

Paoli, A., Bosco, G., Camporesi, E.M., Mangar, D. 2015. Ketosis, ketogenic diet and food intake control, a complex relationship. Front. Psychol. 6: 27. doi.org/10.3389/fpsyg.2015.00027

Park, S., Komatsu, T., Kim, S.E., Tanaka, K., Hayashi, H., Mori, R., Shimokawa, I. 2017a. Neuropeptide Y resists excess loss of fat by lipolysis in calorie-restricted mice: a trait potential for the life-extending effect of calorie restriction. Aging Cell 16: 339–348. doi.org/10.1111/acel.12558

Park, S., Mori, R., Shimokawa, I. 2017b. The fat regulator neuropeptide Y and caloric restriction. Aging 9: 2243–2244.

Rojas, J.M., Bruinstroop, E., Printz, R.L., et al. 2015. Central nervous system neuropeptide Y regulates mediators of hepatic phospholipid remodeling and very low-density lipoprotein triglyceride secretion via sympathetic innervation. Mol. Metab. 4: 210–221. doi.org/10.1016/j.molmet.2015.01.004

Ruiz-Nunez, B., Dijck-Brouwer, D.A., Muskiet, F.A. 2016. The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. J. Nutr. Biochem. 36: 1–20. doi.org/10.1016/j.jnutbio.2015.12.007

Ruohonen, S.T., Vähätalo, L.H., Savontaus, E. 2012. Diet□induced obesity in mice overexpressing neuropeptide y in noradrenergic neurons. Int. J. Pept. 2012: 452524. doi.org/10.1155/2012/452524

Sahu, A., White, J.D., Kalra, P.S., Kalra, S.P. 1992. Hypothalamic neuropeptide Y gene expression in rats on scheduled feeding regimen. Brain Res. Mol. Brain Res. 15: 15–18. doi.org/10.1016/0169-328X(92)90145-2

Santativongchai, P., Fungfuang, W., Boonyawiwat, V., Pongchairerk, U., Tulayakul, P. 2020. Comparison of physicochemical properties and fatty acid composition of crocodile oil (*Crocodylus siamensis*) extracted by using various extraction methods. Int. J. Food Prop. 23: 1465–1474. doi.org/10.1080/10942912.2020.1814324

Sastravaha, A., Suwanna, N., Sinthusingha, C., Noosud, J., Roongsitthichai, A. 2016. Ameliorative effects of omega-3 concentrate in managing coxofemoral osteoarthritic pain in dogs. Thai J. Vet. Med. 46: 305–311.

Scharrer, E. 1999. Control of food intake by fatty acid oxidation and ketogenesis. Nutrition 15: 704–714. doi.org/10.1016/S0899-9007(99)00125-2

Schwartz, M.W., Woods, S.C., Porte, Jr. D., Seeley, R.J., Baskin, D.G. 2000. Central nervous system control of food intake. Nature 404: 661–671. doi.org/10.1038/35007534

Shapiro, A., Strehler, K., Ryder, D., Tümer, N., Scarpace, P.J. 2011. Fructose-induced leptin resistance involves impaired AMPK but intact STAT3 signaling. Appetite. 57: S40.

Shewale, S., Ali, I., Hadawale, K., Bhargava, S. 2018. Response of NPY immunoreactivity in the tadpole brain exposed to energy rich and energy depleted states. Neuropeptides 71: 1–10. doi.org/10.1016/j.npep.2018.06.001

Shim-Prydon, G., Camacho-Barreto, H. 2007. New animal products: New uses and markets for by-products and co-products of crocodile, emu, goat, kangaroo and rabbit. RIRDC Project No. DAQ-320A. A Report for the Rural Industries Research and Development Corporation. Australia.

Tsunekawa, T., Banno, R., Mizoguchi, A., et al. 2017. Deficiency of PTP1B attenuates hypothalamic inflammation via activation of the JAK2-STAT3 pathway in microglia. EBioMedicine 16: 172–183. doi.org/10.1016/j.ebiom.2017.01.007

Ueshima, H., Sekikawa, A., Miura, K., Turin, T.C., Takashima, N., Kita, Y. 2008. Cardiovascular disease and risk factors in Asia: A selected review. Circulation 118: 2702–2709. doi.org/10.1161/CIRCULATIONAHA.108.790048

Venter, T., Fox, L.T., Gerber, M., du Preez, J.L., van Zyl, S., Boneschans, B., du Plessis, J. 2016. Physical stability and clinical efficacy of *Crocodylus niloticus* oil lotion. Rev. Bras. Farmacogn. 26: 521–529. doi.org/10.1016/j.bjp.2016.03.011

Willett, W.C. 2012. Dietary fats and coronary heart disease. J. Intern. Med. 272: 13–24. doi.org/10.1111/j.1365-2796.2012.02553.x

World Health Organization. 2020. Healthy diet. Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/healthy-diet, 28 July 2021.

Zarjevski, N., Cusin, I., Vettor, R., Rohner□Jeanrenaud, F., Jeanrenaud, B. 1993. Chronic intracerebroventricular neuropeptide□Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 133: 1753–1758. doi.org/10.1210/endo.133.4.8404618