The effect of chocolate-based products on some appetite-related hormones: a systematic review

Shirzadi, Z., Djafarian, K., Safabakhsh, M., Clark, C. C. T. & Shab-Bidar, S.

Original citation & hyperlink:
Shirzadi, Z, Djafarian, K, Safabakhsh, M, Clark, CCT & Shab-Bidar, S 2020, 'The effect of chocolate-based products on some appetite-related hormones: a systematic review', International Journal of Food Sciences and Nutrition, vol. 71, no. 7, pp. 785-792. https://dx.doi.org/10.1080/09637486.2020.1734543

DOI 10.1080/09637486.2020.1734543
ISSN 0963-7486
ESSN 1465-3478

Publisher: Taylor and Francis

This is an Accepted Manuscript of an article published by Taylor & Francis in International Journal of Food Sciences and Nutrition, on 04/03/2020, available online: http://www.tandfonline.com/10.1080/09637486.2020.1734543

The effect of chocolate-based products on some appetite-related hormones: A systematic review

Zahra Shirzadi[1], Kurosh Djafarian[1], Cain C. T. Clark[2], Sakineh Shab-Bidar[3]

[1] Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

[2] Centre for Sport, Exercise, and Life Sciences, Coventry University, Coventry, UK, CV15FB

[3]Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

Running title: Chocolate and appetite hormones

Correspondence to:
Sakineh Shab-Bidar, PhD
Associate professor,
Department of Community Nutrition,
School of Nutritional Sciences and Dietetics,
Tehran University of Medical Sciences,
Tel:+98-21- 88955975
Fax:+98-21- 88974462
Email: s_shabbidar@ tums.ac.ir

Abstract

Several studies have shown the effects of chocolate-based products on appetite-related indicators, however, the results of these studies are equivocal. Thus, the aim of the present study was to conduct a systematic review of studies investigating the acute and long-term response of appetite-related hormones to chocolate intake in adults. A systematic search of MEDLINE and EMBASE for published studies, in English, was performed from inception up to November 2018. It appears that ghrelin and leptin are not responsible for the satiating effect of chocolate products. Gastric infusion of milk chocolate elicited a greater increase in cholecystokinin (CCK), in comparison with oral ingestion of milk chocolate and gastric infusion of non-caloric products. Moreover, viscosity seems to have no effect on active CCK and glucagon-like peptide-1. Due to the heterogeneity between studies, limited sample, low quality of evidence, and substantial variation in methods and chocolate products, caution is suggested in interpreting these results.

Introduction

Obesity is an important health issue throughout the world (Organization, 2014, Zijlstra et al., 2009, Larsson et al., 2018). The World Health Organization (WHO) reported that obesity has been doubled since 1980. Indeed, excess body weight has been associated with an increased risk of some chronic diseases such as cardiovascular diseases, type 2 diabetes, and certain cancers (Pi-Sunyer, 1993). In contemporary practice, appetite management is an increasingly common approach used for weight control. Hormones secreted by the gastrointestinal tract regulate the appetite by mediation of hunger and satiety (Karra and Batterham, 2010). In opposition of ghrelin, a number of hormones including cholecystokinin peptide YY (PYY; also known as peptide tyrosine tyrosine), glucagon-like peptide 1 (GLP-1), and pancreatic polypeptide (PP) suppress appetite (Wynne et al., 2005). Previous studies have shown that several foods can play a mediating role in appetite-related hormones (Panahi et al., 2013). Chocolate is a palatable food all over the world; various types of chocolate based products (dark, milk, white chocolate) contain different amounts of macro/micro nutrients (Agriculture, 2010) and flavonoids (Wu et al., 2004). In comparison with milk chocolate, dark chocolate contains a greater proportion of cocoa liquor, and less non-fat cocoa solids (~5-fold greater) (Miller et al., 2009), with the remainder comprising mainly sugar, and a small amount of other components; in addition to the milk added in milk chocolate. White chocolate, on the other hand, is composed of cocoa butter extracted from cocoa liquor, and so it is free from the non-fat cocoa solids that contain flavonoids.

Beneficial health effects of chocolate have been shown in previous studies (Hooper et al., 2012, Larsson et al., 2018). A systematic review and meta-analysis, including 35 randomized controlled trials (RCTs), showed that chocolate-based interventions elicited reductions in body weight, body mass index (BMI) and waist circumference (Kord-Varkaneh et al., 2018). However, it is important to recognize the underlying mechanism of chocolate on weight loss. The effect of chocolate on appetite have been categorized into three type of studies:1) The effect of chocolate on appetite related hormones (Marsh et al., 2017, Zijlstra et al., 2009, Spetter et al., 2014, Zhang et al., 2018, Rigamonti et al., 2015) 2) The effect of chocolate on visual analogue scale (VAS) (Greenberg et al., 2016, Marsh et al., 2017, Smeets et al., 2006, Zhang et al., 2018, Sørensen and Astrup, 2011, Chapelot and Payen, 2010, Harper et al., 2007, Ortinau et al., 2014) 3) The effect of chocolate on

energy intake (Harper et al., 2007, Appleton et al., 2015, Chapelot and Payen, 2010, Greenberg et al., 2016, Marsh et al., 2017, Ortinau et al., 2014, Zijlstra et al., 2009, Zhang et al., 2018). Despite several publications on the effect of chocolate-based products on appetite-related hormones, no study has yet summarized the currently available findings in this regard. Therefore, the purpose of this study was to systematically review the available evidence regarding the effect of chocolate-based products on appetite-related hormones.

Methods

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement was used to conduct this systematic review meta-analysis(Picot et al., 2012) (**Supplementary Table 1**). The PICOS-model (Richardson et al., 1995) was used to formulate the study's question, where the acronym PICOS stands for; Population (all individuals except children under 18 years old and pregnant and lactating women), Intervention(chocolate based products), Comparison (studies which had control group), Outcome (studies that appetite relating hormones concentration), and Study design (RCT, Quasi experimental).

Search strategy

MEDLINE, EMBASE, and PMC were searched for all relevant published articles up to 23 November 2018 with no time restriction. Only articles published in English were considered in this review. We used medical subject headings (MeSH) and text words to identify potential studies. Search words included: ("Chocolate" OR "Cacao" OR "Chocolate*" OR "cocoa" OR "cocoa*" AND "Appetite" OR "Glucagon-Like Peptide 1" OR "Leptin" OR "Peptide YY" OR "Ghrelin OR "appetite hormones"). Of all articles, RCTs were checked by reviewing titles, abstracts, population and study design in order to select relevant publications for inclusion and exclusion criteria. Literature searches were downloaded into EndNote (version X7, for Windows, Thomson Reuters, Philadelphia, PA, USA) to manage and to facilitate the review process.

Eligibility criteria

Two investigators performed an independent search and screening articles. Studies were included if they met the following criteria: a) the study design was interventional, b) the intervention used chocolate based products, c) the outcomes of interest were plasma concentration of appetite-related hormones, such as ghrelin, leptin, Glucagon-Like Peptide 1 "GLP-1", peptide YY "PYY", cholecystokinin "CCK", d) the population of interest was adults (aged >18 years), e) Published English studies, f) oral consumption of chocolate-based product. We excluded studies if they were

observational studies, review articles, duplicated publications, conference papers, animal or cell culture studies and unrelated studies. Any disagreements were discussed and resolved by consensus or by a third independent reviewer (SS-b), if necessary.

Data extraction

The following data from the full text of selected studies were extracted: general characteristics of the study (first author's name, year of publication, the study design, the country where the study was conducted, number of cases and controls, type of cocoa product, total cocoa product dose and duration of follow-up), characteristics of the participants (study population, age, gender) and outcome results (means and standard deviation(SD)s for ghrelin, GLP-1,leptin,CCK and PYY in baseline and after intervention). Since several studies have used figures for showing their outcomes, means and measures of dispersion were approximated from figures in the manuscripts using Web Plot Digitizer (Zhu et al., 2011). Where further detail was required, we contacted study authors for additional information.

Quality assessment

A systematic assessment of bias in the included studies was performed based on the Cochrane Criteria. The quality of the studies evaluated by following criteria: sequence generation sufficiency, allocation concealment, blinding, elucidating of dropouts (imperfect outcome data), selective outcome reporting, and other possible sources of bias. According to Cochrane Handbook (Higgins, 2011) recommendation, studies were stratified as low risk of bias, high risk of bias or unclear regarding each domain (**Figure 1**).

Results

Study selection

The flow diagram of literature search is shown in **Figure 2**. The initial search identified 217 publications. Of those, 177 records were excluded based on title and abstract screening. The cited references of the retrieved articles were checked to find any potential eligible studies; consequently, we found one other related article through backward-searching for references cited in those articles, after screening for title and abstract. Of 40 full texts screened, 35 articles were excluded due to a lack sufficient data.

Characteristics of included studies

Characteristics of the included studies are summarized in **Table 1**. They were published between 2009 and 2018. The duration of follow-up in these studies was from 30 to 180 minutes after

chocolate-based product consumption. Four studies had a crossover design (Zhang et al., 2018, Marsh et al., 2017, Zijlstra et al., 2009, Spetter et al., 2014) and one was within-subject repeated-measure study (Rigamonti et al., 2015). The participants of two studies consisted of both genders (Zijlstra et al., 2009, Zhang et al., 2018), one study was conducted on women only (Marsh et al., 2017), and Rigamonti et al. and Spetter et al. was conducted on men only (Rigamonti et al., 2015, Spetter et al., 2014). The cases received dark/white/milk chocolate and chocolate with a milky creamy filling in doses of less or more than 100 g per session. The cocoa content of chocolate products ranged from 0 to 80%. Chocolate with a milky creamy filling and chocolate with a milky creamy filling and puffed cereal had 40% cocoa in = Zhang et.al (Zhang et al., 2018). In Marsh et.al, dark chocolate, milk chocolate, and white chocolate contained 80%, 35% and 0% cocoa, respectively (Marsh et al., 2017). Both arms of Zijlstra et,al study included milk chocolate with 1.5% cocoa (Zijlstra et al., 2009), Spetter et al. used a commercially available full-fat chocolate milk (Spetter et al., 2014), whilst another study used a milk chocolate tablet, but the cocoa content was not record (Rigamonti et al., 2015). A large variation in energy content of test foods was observed.

Ghrelin

All five studies reported ghrelin concentration within 2.5 to 180 min after consumption of chocolate products, and in most studies, changes were similar to control groups. Zhang et al. conducted a cross-over experimental study on 20 volunteers (10 men and 10 women) to evaluate the effects of chocolate based products consumption on ghrelin during 180 min and 21 days intervention. Participants were split into four groups: the purified water group [blank group]; the tested biscuit group (250 kcal [reference group]; the chocolate with milky creamy filling group (3.5 bars/day, 12.5 g per bar), or the chocolate with milky creamy filling and puffed cereal group (2 bars/day, 23.5 g per bar). Zhang et al found that the serum ghrelin levels were similar across the water group, tested biscuit group, chocolate with milky creamy filling group and chocolate with milky creamy filling and puffed cereal group. The percent change in ghrelin was not significant among the four test food groups at any time point (Zhang et al., 2018). Zijstra et al. conducted a cross-over study on 32 subjects to examine the effects of a fixed amount of a chocolate flavored milk-based liquid or semi-solid product, similar in energy density and macronutrient composition, on some appetite-related hormones. The authors concluded that active ghrelin concentrations decreased after the intake of the chocolate product, however, there was no differences over time

points between the liquid and the semi-solid test product (Zijlstra et al., 2009). Another study by Rigamonti et al., conducted on ten satiated severely obese subjects, examined the role of some gastrointestinal peptides following chocolate consumption. Indeed, circulating levels of ghrelin decreased significantly over time in both the milk-chocolate (palatable) and bread and butter group (non-palatable). However, area under the curve (AUC) analysis highlighted that circulating levels of ghrelin in the palatable group was more than the non-palatable group (Rigamonti et al., 2015). Another study compared the acute effect of consuming an isocaloric dose of dark, milk and white chocolate on subsequent energy intake, appetite, and mood in fourteen healthy postmenopausal women. There was no significant difference in ghrelin values between dark, milk, and white chocolate groups at 30 or 90 minutes, respectively (Marsh et al., 2017). Spetter et al., in a randomized, single-blinded, cross-over experiment, determined the contributions of gastric and oral stimulation to the appetite and hormone responses, and their effect on ad libitum intake. Fourteen healthy male subjects were randomized to 3 treatment sessions:1) naso-gastric infusion of 500 mL/0 kJ water, 2) naso-gastric infusion of 500 mL/1770 kJ chocolate milk, and 3) oral administration of 500 mL/1770 kJ chocolate milk. Measurements at fixed time points, up to 30 min after infusion or oral administration, indicated that total ghrelin AUC was significantly higher for oral consumption compared to the gastric administration (Spetter et al., 2014).

GLP-1

Two studies included in this review reported the concentration of GLP-1 (Rigamonti et al., 2015, Zijlstra et al., 2009). No significant changes in GLP-1 concentration were observed in Rigamonti et al. following intake of chocolate or a non-palatable alternative at breakfast (Rigamonti et al., 2015). Zijlstra et al. reported an increase in GLP-1 level after intake of liquid and semi-solid chocolate, however, there was no significant difference between liquid and semi-solid chocolate products (Zijlstra et al., 2009).

CCK

Two studies evaluated the effects of chocolate intake on CCK-8. Zijlstra et al. observed an increase in CCK, although, there was no significant difference between liquid and semi-solid products (Zijlstra et al., 2009). Spetter et al. showed that time and type of feeding may be an important issue in determining of CCK-8 concentration, where CCK-8 level was significantly higher at 2.5 and 5 min after the gastric exposure of chocolate, as compared to the oral exposure to chocolate; whilst

10 minutes after exposure, CCK-8 value was significantly higher in the gastric feeding, as compared than the oral feeding(Spetter et al., 2014).

PYY

Only one study reported changes of PYY after the intake of chocolate. Rigamonti et al. showed that circulating levels of PYY changed significantly over time, but not between exposure sessions. Indeed, they found that administration of both breakfast-time chocolate or bread and butter elicited a significant increase in circulating concentration of PYY at 130 min, 160 min, and 190 min, respectively.

Leptin

Marsh et al. evaluated the acute effect of consuming an isocaloric dose of dark, milk, and white chocolate on subsequent energy intake, appetite, and mood in postmenopausal women, and observed no differences in leptin level before or after chocolate consumption (Marsh et al., 2017).

PP

Marsh et al. showed that dark and milk chocolate elicited a greater elevation in PP concentration as compared to white chocolate (Marsh et al., 2017).

Discussion

To the best of our knowledge, this is the first systematic review to have examined the role of chocolate-based products on appetite-related hormones. Previous studies have shown that several hormones are associated with appetite; for example, an elevations in ghrelin concentration acts as a signal to commence eating behavior (Callahan et al., 2004). However some other hormones, such as CCK, leptin, GLP-1, PYY, insulin, and PP have been associated with appetite suppression (Wren and Bloom, 2007).

As commercially-available chocolates are high in calories, simple sugar, and fat (Greenberg et al., 2016), they may contribute to excess energy intake, which may lead to weight gain in the long-term (Dehghan et al., 2017); indeed, Greenberg et al. highlighted that frequent chocolate consumption is associated with greater weight gain over the long term (Greenberg and Buijsse, 2013). A recent dose response meta-analysis concluded that cocoa/dark chocolate supplementation

does not affect body weight, BMI and WC in comparison with control group (Kord-Varkaneh et al., 2018). However, the authors did report that ≥ 30g/d chocolate supplementation, for between four to eight weeks, resulted in weight and BMI reductions (Kord-Varkaneh et al., 2018).

There is some evidence to support the hypothesis that ghrelin has a role in long term weight gain regulation (Cummings, 2006). Indeed, it has been asserted that ghrelin, leptin, and GLP-1 are not responsible for differences in the satiating effect of test foods, where no difference in circulating concentrations of these peptides has been reported between experimental conditions (Marsh et al., 2017, Spetter et al., 2014, Zhang et al., 2018, Rigamonti et al., 2015). Differences in viscosity has been reported to confer a significant effect on desacyl ghrelin (Zijlstra et al., 2009), whilst gastric infusion of chocolate milk decreased total ghrelin concentrations to a greater extent as compared to normal ingestion, although no differences in active ghrelin response were reported between conditions in Spetter et al (Spetter et al., 2014). In the present study, CCK changes were different between the varying conditions of included studies. In Spetter et al, a greater elevation in CCK-8 level over 30 min after gastric infusion of chocolate milk was observed in comparison with oral ingestion (Spetter et al., 2014). Further, Spetter et al suggested that higher gastric emptying rate after gastric infusion of chocolate milk compared with oral ingestion can elicit a greater elevation in CCK-8 (Spetter et al., 2014). PP was reportedly increased in a dose-response manner with the polyphenol content of the chocolate in one study (Marsh et al., 2017). Indeed, there is some evidence to suggest that polyphenols can influence some hormones in response to food intake (Panickar, 2013). In the present review, just one study assessed PYY, and although PYY level was increased following the consumption of both milk chocolate and bread and butter, respectively, there was no significant difference between two groups (Rigamonti et al., 2015). It is conceivable that the gastric emptying rate of liquid chocolate products could be faster compared to semi-solid products. Indeed, studies have shown that increasing the viscosity of food decreases the gastric emptying rate (Zhu et al., 2013, Moxon et al., 2017); given the slower gastric emptying rates of the more viscous product, it is possible that it took more time before nutrients of the semi-solid product reached the duodenum and the rest of the small intestine. Since ghrelin is produced primarily by the stomach and proximal small intestine (Cummings and Overduin, 2007), this could explain why the levels for desacyl ghrelin are higher after intake of semi-solid products. This is also in agreement with the study of Blom et al, where it was demonstrated that postprandial ghrelin responses are inversely associated with gastric emptying rate(Blom et al., 2006). However, it is

not known if the gastric emptying rate was indeed different between the products. Furthermore, it is unclear, yet, if the hormone responses to chocolate-based products are influenced by energy content, ingredients of tested foods, the viscosity of foods, cocoa content, or the foods texture, and thus represent viable avenues for further research.

Limitations

This study has some limitations. Our search was limited to those articles published in English only, and thus, we may have inadvertently missed some potentially relevant studies. Another limitation is the variation in the composition and amount of chocolate-based products, duration, viscosity, type of nourishment, time of consumption of last meal, age, and BMI of participants. The authors recommend that the texture of chocolate-based products be assessed in future studies to discern whether it has an influence on hormonal responses.

Conclusion

Published studies of the effect of chocolate products on appetite-related hormones have substantial variation in methods and type. Some hormones were only assessed in singular studies, whilst little or no differences in appetite-related hormones were reported in many studies. Thus, to aid decision-making, well-controlled RCTs, with longer intervention periods, are needed to elucidate the effect of chocolate-based products on appetite-related hormones.

Funding sources

The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no potential conflict of interest.

Acknowledgment

ZS designed this study. KD and SS-b supervised the study. ZS conducted the literature searches, data extraction and independent search and reviewing. ZS prepared a first draft of the manuscript, and SS-b and KD finalized it.

References

AGRICULTURE, U. S. D. O. 2010. USDA National nutrient database for standard reference, release 28. Agricultural Research Service.

APPLETON, K. M., MCKEOWN, P. P. & WOODSIDE, J. V. 2015. Energy compensation in the real world: good compensation for small portions of chocolate and biscuits over short time periods in complicit consumers using commercially available foods. *Appetite,* 85**,** 104-110.

BLOM, W. A., LLUCH, A., VINOY, S., STAFLEU, A., VAN DEN BERG, R., HOLST, J. J., KOK, F. J. & HENDRIKS, H. F. 2006. Effects of gastric emptying on the postprandial ghrelin response. *American Journal of Physiology-Endocrinology and Metabolism,* 290**,** E389-E395.

CALLAHAN, H. S., CUMMINGS, D. E., PEPE, M. S., BREEN, P. A., MATTHYS, C. C. & WEIGLE, D. S. 2004. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *The Journal of Clinical Endocrinology & Metabolism,* 89**,** 1319-1324.

CHAPELOT, D. & PAYEN, F. 2010. Comparison of the effects of a liquid yogurt and chocolate bars on satiety: a multidimensional approach. *British journal of nutrition,* 103**,** 760-767.

CUMMINGS, D. E. 2006. Ghrelin and the short-and long-term regulation of appetite and body weight. *Physiology & behavior,* 89**,** 71-84.

CUMMINGS, D. E. & OVERDUIN, J. 2007. Gastrointestinal regulation of food intake. *The Journal of clinical investigation,* 117**,** 13-23.

DEHGHAN, M., MENTE, A., ZHANG, X., SWAMINATHAN, S., LI, W., MOHAN, V., IQBAL, R., KUMAR, R., WENTZEL-VILJOEN, E. & ROSENGREN, A. 2017. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *The Lancet,* 390**,** 2050-2062.

GREENBERG, J. A. & BUIJSSE, B. 2013. Habitual chocolate consumption may increase body weight in a dose-response manner. *PLoS One,* 8**,** e70271.
GREENBERG, J. A., O'DONNELL, R., SHURPIN, M. & KORDUNOVA, D. 2016. Epicatechin, procyanidins, cocoa, and appetite: a randomized controlled trial. *The American journal of clinical nutrition,* 104**,** 613-619.
HARPER, A., JAMES, A., FLINT, A. & ASTRUP, A. 2007. Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. *British Journal of Nutrition,* 97**,** 579-583.
HIGGINS, J. 2011. Cochrane handbook for systematic reviews of interventions. Version 5.1. 0 [updated March 2011]. The Cochrane Collaboration. *www. cochrane-handbook. org*.
HOOPER, L., KAY, C., ABDELHAMID, A., KROON, P. A., COHN, J. S., RIMM, E. B. & CASSIDY, A. 2012. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *The American journal of clinical nutrition,* 95**,** 740-751.
KARRA, E. & BATTERHAM, R. L. 2010. The role of gut hormones in the regulation of body weight and energy homeostasis. *Molecular and cellular endocrinology,* 316**,** 120-128.
KORD-VARKANEH, H., GHAEDI, E., NAZARY-VANANI, A., MOHAMMADI, H. & SHAB-BIDAR, S. 2018. Does cocoa/dark chocolate supplementation have favorable effect on body weight, body mass index and waist circumference? A systematic review, meta-analysis and dose-response of randomized clinical trials. *Critical reviews in food science and nutrition*.
LARSSON, S. C., DRCA, N., JENSEN-URSTAD, M. & WOLK, A. 2018. Chocolate consumption and risk of atrial fibrillation: Two cohort studies and a meta-analysis. *American heart journal,* 195**,** 86-90.
MARSH, C. E., GREEN, D. J., NAYLOR, L. H. & GUELFI, K. J. 2017. Consumption of dark chocolate attenuates subsequent food intake compared with milk and white chocolate in postmenopausal women. *Appetite,* 116**,** 544-551.
MILLER, K. B., HURST, W. J., FLANNIGAN, N., OU, B., LEE, C., SMITH, N. & STUART, D. A. 2009. Survey of commercially available chocolate-and cocoa-containing products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao. *Journal of agricultural and food chemistry,* 57**,** 9169-9180.
MOXON, T. E., NIMMEGEERS, P., TELEN, D., FRYER, P. J., VAN IMPE, J. & BAKALIS, S. 2017. Effect of chyme viscosity and nutrient feedback mechanism on gastric emptying. *Chemical engineering science,* 171**,** 318-330.
ORGANIZATION, W. H. 2014. http://www. who. int/mediacentre/factsheets/fs340/en. Accessed.
ORTINAU, L. C., HOERTEL, H. A., DOUGLAS, S. M. & LEIDY, H. J. 2014. Effects of high-protein vs. high-fat snacks on appetite control, satiety, and eating initiation in healthy women. *Nutrition journal,* 13**,** 97.
PANAHI, S., LUHOVYY, B. L., LIU, T. T., AKHAVAN, T., EL KHOURY, D., GOFF, H. D. & ANDERSON, G. H. 2013. Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults. *Appetite,* 60**,** 154-161.
PANICKAR, K. S. 2013. Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity. *Molecular nutrition & food research,* 57**,** 34-47.
PI-SUNYER, F. X. 1993. Medical hazards of obesity. *Annals of internal medicine,* 119**,** 655-660.
PICOT, J., HARTWELL, D., HARRIS, P., MENDES, D., CLEGG, A. & TAKEDA, A. 2012. The preferred reporting items for systematic reviews and meta-analyses checklist.
RICHARDSON, W. S., WILSON, M. C., NISHIKAWA, J. & HAYWARD, R. S. 1995. The well-built clinical question: a key to evidence-based decisions. *ACP journal club,* 123**,** A12-A12.

RIGAMONTI, A. E., PISCITELLI, F., AVETA, T., AGOSTI, F., DE COL, A., BINI, S., CELLA, S. G., DI MARZO, V. & SARTORIO, A. 2015. Anticipatory and consummatory effects of (hedonic) chocolate intake are associated with increased circulating levels of the orexigenic peptide ghrelin and endocannabinoids in obese adults. *Food & nutrition research,* 59**,** 29678.

SMEETS, P. A., DE GRAAF, C., STAFLEU, A., VAN OSCH, M. J., NIEVELSTEIN, R. A. & VAN DER GROND, J. 2006. Effect of satiety on brain activation during chocolate tasting in men and women. *The American journal of clinical nutrition,* 83**,** 1297-1305.

SØRENSEN, L. B. & ASTRUP, A. 2011. Eating dark and milk chocolate: a randomized crossover study of effects on appetite and energy intake. *Nutrition & diabetes,* 1**,** e21.

SPETTER, M. S., MARS, M., VIERGEVER, M. A., DE GRAAF, C. & SMEETS, P. A. 2014. Taste matters–effects of bypassing oral stimulation on hormone and appetite responses. *Physiology & behavior,* 137**,** 9-17.

WREN, A. & BLOOM, S. 2007. Gut hormones and appetite control. *Gastroenterology,* 132**,** 2116-2130.

WU, X., BEECHER, G. R., HOLDEN, J. M., HAYTOWITZ, D. B., GEBHARDT, S. E. & PRIOR, R. L. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of agricultural and food chemistry,* 52**,** 4026-4037.

WYNNE, K., STANLEY, S., MCGOWAN, B. & BLOOM, S. 2005. Appetite control. *Journal of Endocrinology,* 184**,** 291-318.

ZHANG, C.-X., LONG, W.-Q., YE, Y.-B., LU, M.-S., ZHANG, N.-Q., XU, M., HUANG, J. & SU, Y.-X. 2018. Effects of chocolate-based products intake on blood glucose, insulin and ghrelin levels and on satiety in young people: a cross-over experimental study. *International journal of food sciences and nutrition,* 69**,** 882-891.

ZHU, Y., HSU, W. H. & HOLLIS, J. H. 2013. The impact of food viscosity on eating rate, subjective appetite, glycemic response and gastric emptying rate. *PLoS One,* 8.

ZHU, Y., XIA, M., YANG, Y., LIU, F., LI, Z., HAO, Y., MI, M., JIN, T. & LING, W. 2011. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clinical chemistry,* 57**,** 1524-1533.

ZIJLSTRA, N., MARS, M., DE WIJK, R. A., WESTERTERP-PLANTENGA, M. S., HOLST, J. J. & DE GRAAF, C. 2009. Effect of viscosity on appetite and gastro-intestinal hormones. *Physiology & Behavior,* 97**,** 68-75.

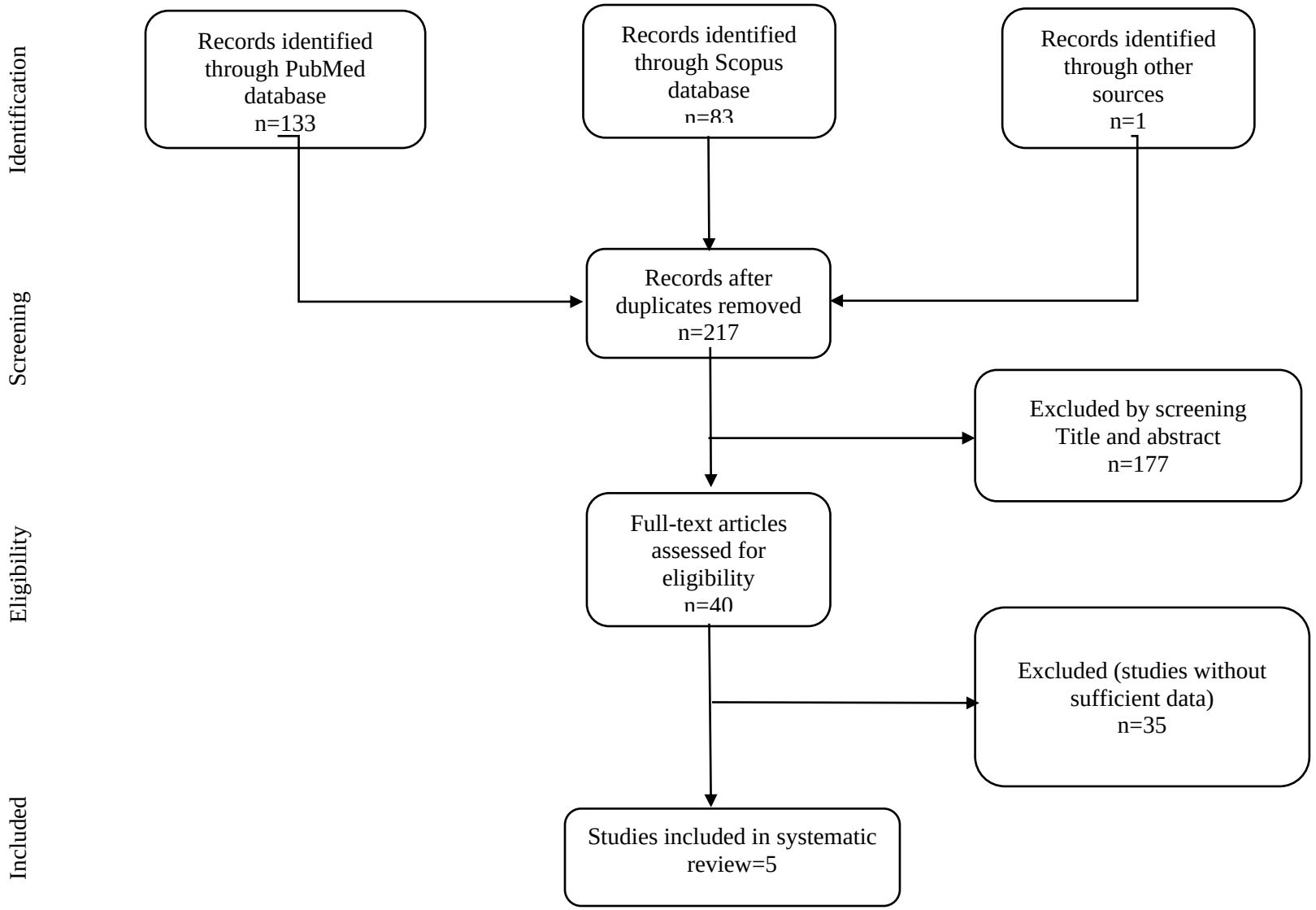

Figure 2. Flow diagram of article screening

Table1. Characteristics of included studies

Author, year	Country	Design	N	Sex	Age (year)	BMI	Study arms	Dose (g or ml)	(% of cocoa)	Energy (Kcal)	P[4],C[5],F[6],S[7] (g)	Duration(min)	Results	Outcome
Zhang, 2018	China	Cross-over	20	both	24.5	21	Purified water	43.75	-	0	-	60,120,180[1]	No difference in ghrelin concentrations among the four test food groups at any time point.	Ghrelin
							Tested biscuit	47.8	0	250	3.9,13.2,28.9,8.2			
							Chocolate with milky creamy filling	43.75	40	247	3.8,15.2,23.4,21.3			
							Chocolate with milky creamy filling +puffed cereal	47	40	262	4,15.7,25.8,25			
Marsh, 2017	Australia	Cross-over	14	female	57.6	24.3	Dark chocolate	84	80	499.76,	6.5, 30.3, 30.4, 16.2	30,90	No difference in the response of ghrelin or leptin to chocolate consumption between conditions (P > 0.05). Higher elevation in PP in response to dark and also milk chocolate compared with white chocolate.	Leptin Ghrelin pp
							Milk chocolate	87	35	480.40,	6.1, 37.0, 29.5, 31.0			

							White chocolate	85	0	501.67	4.1,37.5,28.9,36.1			
Rigamonti, 2015	Italy	Quasi experimental	10	male	33.9	42.6	Milk chocolate	200	-	1000	19.7,160,34,-	30,60,90,120	Elevation in ghrelin was higher after the ingestion of chocolate in comparison with bread and butter GLP-1 didn't changed significantly. PYY changed significantly over the sampling times, but not between the two experimental sessions of eating.	Ghrelin GLP-1 PYY
							Bread and butter	-	0	1000	Same with control			
Zijlstra, 2009	Netherlands	Cross-over	32	both	22	21.9	Milk chocolate (semi solid)	M:460 W:358	1.5	M:461 W:359	M:13.3,64.4,16.5,- W:10.0,50.1,12.8	30,60,90	A similar response of ghrelin, CCK-8 and GLP-1 after a fixed amounts of a liquid and semi-solid milk chocolate. A statistically significant product effect for desacyl ghrelin. (The values after consumption of the semi-solid product were consistently higher than the values after the liquid product)	Ghrelin GLP-1 CCK
							Milk chocolate (liquid)	M:480 W:380	1.5	M:460 W:368	M:13.4,66.7,16.3 W:10.0,50.4,12.9			

Spetter , 2014	Netherlands	Cross-over	14	male	24.6	22.3	Milk chocolate	500 ml	-	422.7	17.5,60,12.5	2.5,5,10,15,30	Gastric infusion of a caloric load increased CCK-8 and decreased total ghrelin concentrations more than ingestion but no differences in active ghrelin response were observed between conditions. In all 3 conditions the CCK-8 concentrations were significantly different.	Ghrelin CCK
							Water + guar gum	500ml	0	0	0			

1- Ghrelin was measured after 21 day, too; 2- Did not include in meta-analysis because of unclear data; 3- C: Chocolate 4-protein; 5-carbohydrate; 6-fat; 7-sugar