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**THE SHORT-TERM IMPACT OF DIETARY FAT AND SUGAR INTAKE ON BREAST MILK COMPOSITION: THE DIET AND BREAST MILK COMPOSITION STUDY**

**Protocol version:** 1.0

**Date:** 21th February 2018

**Study registration:**

To register Australian and New Zealand Clinical Trial Registry

**Funding:**

The University of Adelaide

**Chief Investigator:**

Associate Professor Beverly Muhlhausler (BM)

**Sponsoring Institution:**

The University of Adelaide

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

I agree that the study will be conducted in accordance with the conditions outlined in the protocol (subject to any amendments). I have read and understood the protocol.

**Investigator’s Name: Beverly Muhlhausler**

**Investigator’s Signature: ……**

**Date:**

GLOSSARY OF ABBREVIATIONS

AGHE Australian Guide to Healthy Eating

BMI Body Mass Index

CRF Case Report Form

GCP Good Clinical Practice

GLP-1 Glucagon-like peptide-1

HREC Human Research Ethics Committee

SAHMRI South Australian Medical Research Institute

WCH Women’s and Children’s Hospital

WCHN Women’s and Children’s Health Network

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# SYNOPSIS

**Title:** The short-term impact of dietary fat and sugar intake on breast milk composition

**Objective:** To assess the effect of breast feeding women consuming a high-fat or high-sugar breakfast on concentrations of metabolic hormones (leptin, insulin, adiponectin, ghrelin and GLP-1) and key macronutrients (fat, protein, lactose) in their breast milk over the subsequent 12 hours.

**Design :** Open label crossover design

**Outcomes**

**Primary:** Breast milk concentrations of metabolic hormones (leptin, insulin, adiponectin, ghrelin and GLP-1) in the 12 hours following consumption of either a high-fat or high-sugar breakfast compared with a control breakfast.

**Secondary:** Breast milk concentrations of macronutrients (fat, protein, lactose) in the 12 hours following consumption of either a control, high-fat or high-sugar breakfast.

**Study duration:** The study will be conducted on three non-consecutive days. Women will receive each breakfast meal once in a random order.

**Interventions:** On the three days of the intervention, women will receive (in random order) a breakfast meal containing either the fat and sugar content consistent with the Australian Guide to Healthy Eating (12g fat, 25g of sugar) or a breakfast meal containing higher levels of fat (28g fat, 18g of sugar) or sugar (5g fat, 57g of sugar). All breakfast meals will be similar in composition (cereal, milk, yogurt, toast and spread) and will be matched for total energy as closely as possible.

**Participants:** 25 breast feeding women

# 2. INVESTIGATORS AND FACILITIES

## 2.1. Investigators

**Principal investigator**

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## 2.2 Roles and responsibilities:

BM - will have overall responsibility for the management of the study, and ensuring adherence to Good Clinical Practice (GCP) [1] and relevant SOPs by all research staff and students involved in the study. BM will have primary responsibility for monitoring study progress. She will also oversee the macronutrients and metabolic hormones analyses of the breast milk samples collected in the study.

MN - will work closely with BM in managing the study, ensuring adherence to Good Clinical Practice (GCP) [1] and relevant SOPs by all research staff and students involved and monitoring study progress. MN will have also oversee the design of the dietary interventions in the study.

GEL – will assist with participant’ recruitment, completion of CRF, sample collection and will perform all macronutrients and metabolic hormones analyses of collected breast milk samples.

## 2.3 Study locations

*Dietary intervention and sample collection*

Healthy Mothers, Babies and Children Theme Clinic Rooms

SAHMRI at WCHN

Level 7, Rieger Building, Women’s and Children’s Hospital

King Willam Road, North Adelaide

SA 5006

Samuel Way Building, Women’s and Children’s Hospital

King Willam Road, North Adelaide

SA 5006

*Advertising and participant recruitment*

Women’s and Children’s Hospital

King Willam Road, North Adelaide

SA 5006

South Australian Health and Medical Research Institute (SAHMRI)

North Terrace, Adelaide

SA 5001

Community Centres

*Breast Milk Analysis*

South Australian Health and Medical Research Insistute (SAHMRI)

North Terrace, Adelaide

SA 5001

Food and Nutrition Research Laboratories

University of Adelaide – Waite Campus

Waite Road, Urrbrae, SA 5064

# INTRODUCTION AND BACKGROUND

Breast milk is uniquely designed for the human infant and contains all the nutrients and bioactive factors required to support optimal infant health and development [2]. Knowledge of breast milk composition has increased significantly in recent years and it is now clear that, in addition to macronutrients (fat, protein and carbohydrates), breast milk also contains a wide range of other bioactive compounds, including metabolic hormones involved in regulating appetite, metabolism and gut function [3]. These metabolic hormones have been shown to be transferred to the breast-fed infant, and the concentrations of several of these factors have been consistently related to measures of infant growth and fat deposition [4, 5].

While it is increasingly clear that there are significant variations in the concentrations of both macronutrients and metabolic hormones in breast milk between individual women, our understanding of the factors that determine these concentrations is limited. Maternal diet is often regarded as a critical determinant of breast milk composition [3], yet evidence-based information on the relationship between dietary intakes in breast feeding women and the composition of their breast milk is surprisingly sparse. Indeed, with the possible exception of fat content and fatty acid composition, the relationship between specific dietary components and the levels of macronutrients and bioactive hormones in the breast milk remains unclear [16]. This information has clinical significance, since it will inform the extent to which dietary interventions introduced during lactation have the potential to modulate breast milk composition, and therefore potentially improve the long-term health outcomes of the child.

Therefore, the aim of this study is to address this knowledge gap by determining the impact of providing breast-feeding women with a breakfast containing differing amounts of fat and sugar on the post-prandial concentrations of macronutrients and metabolic hormones in their breast milk over the subsequent 12-hour period.

# STUDY OBJECTIVES

## Main objective

To assess the effect of breast feeding women consuming a high-fat or high-sugar breakfast on concentrations of metabolic hormones (leptin, insulin, adiponectin, ghrelin and glucagon-like peptide-1 (GLP-1)) and key macronutrients (fat, protein, lactose) in their breast milk over the subsequent 12 hours.

## Specific objectives

**Primary:** To assess and compare breast milk concentrations of metabolic hormones (leptin, insulin, adiponectin, ghrelin and GLP-1) in the 12 hours following consumption of either a high-fat or high-sugar breakfast compared with a control breakfast.

**Secondary:** To assess changes in breast milk concentrations of macronutrients (fat, protein, lactose) in the 12 hours following consumption of either a control, high-fat or high-sugar breakfast.

# METHODS: Participants, interventions, and outcomes

## 5.1 Study design

This study will be an open label crossover design with women assigned to receive the high-fat, high-sugar and control breakfasts in a random order. There will be a one-week wash-out period between the different breakfasts.

## 5.2 Study setting

This study will be performed in the Healthy Mothers, Babies and Children Theme clinic rooms at South Australian Health and Medical Research Institute (SAHMRI) at WCHN (Level 7, Rieger Building, Women’s and Children’s Hospital) and Samuel Way Building at the Women’s and Children’s Hospital.

Women will be screened for eligibility either antenatally or postnatally, either in the Women’s and Children’s Hospital, at SAHMRI or in community centres.

## Eligibility criteria

### 5.3.1 Inclusion Criteria

- Singleton pregnancy

- Term delivery

- Women who are currently breastfeeding

- Mothers between 6 weeks and 16 weeks post-partum at the time of the first study visit

### 5.3.2 Exclusion Criteria

- Known major congenital abnormalities or health issues in the infant that could significantly affect feeding behavior

- Complimentary feeding introduced before first study session

- Maternal diseases known to affect gastric absorption

- Maternal diabetes

- Restrictive diets (e.g. gluten free, dairy free, milk free, vegan)

## Interventions

The dietary interventions will be provided in the form of a specially designed breakfast meal which either aligns with the fat and sugar content consistent with the Australian Guide to Healthy Eating (AGHE) (control, 12g fat and 25g sugar) or higher levels of fat (high-fat, 28g fat) or higher levels of sugar (high-sugar, 57g sugar). The breakfast meal will be provided at ~7:30am after women have fasted from midnight on the previous day. The breakfast meals will contain similar foods (cereal, milk, yogurt, toast and spread) and will be matched for total energy content as closely as possible.

### High-fat Breakfast Meal

The high-fat breakfast meal will consist of: 30g Muesli, 100ml full fat milk, 90g Greek yogurt, 42.5g (1 slice) soy and linseed bread and 8g butter. This breakfast provides 28g of fat, 18g of sugar and has an energy content of 2161 kJ.

### High-sugar Breakfast Meal

The high-sugar breakfast meal will consist of: 30g high-fibre breakfast cereal, 100ml semi-skimmed milk, 100g strawberry yogurt, 65g (1 slice) fruit bread, 15g raspberry jam, 1 teaspoon honey and 1 teaspoon of white sugar. This breakfast provides 5g of fat, 57g of sugar and has an energy content of 2100 kJ.

### Control (AGHE) Breakfast Meal

The control breakfast meal will consist of: 45g whole grain cereal, 200ml semi-skimmed milk, 160g vanilla low-fat yogurt, 1 wholemeal crumpet, 70g fresh strawberries and 8g butter. This breakfast provides 12g of fat, 25g of sugar and has an energy content of 2132 kJ.

**Energy ratio**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Breakfast meal | Protein (%) | Fat (%) | Saturated fat (%) | Carbohydrate (%) | Sugar (%) |
| High in fat | 14 | 48 | 23 | 35 | 14 |
| High in sugar | 13 | 9 | 4 | 75 | 45 |
| Control | 23 | 20 | 11 | 52 | 20 |

## Outcomes

**Primary Outcome**

Breast milk concentrations of metabolic hormones (leptin, insulin, adiponectin, ghrelin and GLP-1) in the 12 hours following consumption of either a control (AGHE), high-fat or high-sugar breakfast.

**Secondary Outcome**

Breast milk concentrations of macronutrients (fat, protein, lactose) in the 12 hours following consumption of either a control (AGHE), high-fat or high-sugar breakfast.

## 5.6 Timeline and procedures

**Screening and Recruitment:** We will recruit antenatally or postnatally for this study. Women will be approached and provided with a brief outline of the study. Women indicating an interest in the study will be screened for eligibility by asking a series of yes/no questions related to the study inclusion/exclusion criteria (see Screening Consent form).

Interested women who are screened antenatally or prior to 5 weeks post-partum and are deemed eligible to participate based on the information provided will be asked to sign a Screening Consent form and Consent to Contact Form, providing consent for us to contact them after delivery. Interested women who are screened postnatally can be enrolled into the study as soon as eligibility is confirmed.

Women who sign the Screening Consent and Consent to Contact form will be contacted at ~5 weeks post-partum and their eligibility to participate will be reconfirmed. Eligible women who are still interested in participating will be provided with further details of the study and given the opportunity to discuss the study with family/friends and ask the research staff any questions they may have about the study.

**The study visits**

Women who are interested and eligible for the study will be invited to attend three separate sessions at the Women’s and Children’s Hospital, with at least one week between sessions. At each session, women will be asked to fast from midnight on the day before and to arrive for the study visit at ~7:00 in the morning of the session. A baseline breast milk sample will be collected and women will then be provided with either a high-fat, high-sugar or control breakfast meal (in random order determined by a computer-based approach). The women will then be asked to remain in or around the Women’s and Children’s Hospital for meals during the day. A TV, wi-fi and a selection of books and magazines will be provided for the women, as well facilities for baby changing and heating bottles for those that are feeding expressed breast milk or are partially formula feeding. The participants will be provided with morning tea, lunch, afternoon tea and snacks throughout the day. The nutrient profile of the meals and snacks will be consistent with the AGHE. Women will be asked to record everything they eat during the day. Breast-milk samples will be collected at regular intervals throughout the day (1 hour, 2 hours, 4 hours and 6 hours after the breakfast meal) for the assessment of macronutrients and metabolic hormone concentrations. Following the collection of the 6-hour sample, women will be able to return home, where they will be asked to collect a further breast milk sample at ~8pm (~12 hours after the breakfast meal). Where women are not able to attend a study session at the Women’s and Children’s Hospital, the breakfast foods, snacks and lunch can be provided to the women in her own home.

At the time of booking the first session women will be asked to provide details of any food allergies or intolerances, so that their breakfast meals can be adjusted accordingly if required. This information will be record in the study Case Report Form and documented in the study database to ensure that this information is readily accessible.

Women will be given $50 at the end of each session to off-set expenses associated with taking part in this study (such as care for siblings, travel and car parking).

Participant contact/sample collection

### 5.6.1 Baseline

Baseline clinical and sociodemographic characteristics of the mother including age, education, household income, alcohol intake, smoking status, use of dietary supplements and presence of chronic diseases will be collected by study research staff into the Case Report Form at the first clinic session. If women report that they are taking dietary supplements and/or medications (other than supplements that are designed specifically for pregnancy/breastfeeding) they will be provided with details of the Medicines Information Centre in SA, where they can confirm that the supplements/medications are safe for use during pregnancy, if they have not already done so. Body weight and height of the women will also be measured. Infant information, including sex, gestational age at birth, birth weight and length, current supplements, medications or chronic health conditions will be obtained by parent report and infant weight and length measured by study staff. Women will be asked to complete a standardised food frequency questionnaire at their first study visit and a 24-hour diet record at each study visit describing intake on the day prior to the study breakfast.

### 5.6.2 Sample collection during the study

At each of the three sessions, women will be asked to collect breast milk (~5-10ml) at baseline (after arrival at the WCH and before eating breakfast) and at 1 hour, 2 hours, 4 hours and 6 hours after breakfast. Breast milk samples (foremilk) will be collected from the breast opposite to the one used to feed the infant at the previous feed. Women will be reminded of the timing of sample collection by study staff and will be given a private area in which they will be able to express breast milk either manually or using a hand breast pump (provided).

Women will be also asked to collect breast milk samples ~12 hours after the breakfast (~8pm). Breast milk samples collected at home can be stored in their home freezer. Women will be provided with an esky and ice brick to bring samples to their next clinic session, and we will arrange collection of samples from their final session from the women’s home by study staff.

### 5.6.3 Summary of Data to be collected

- 6 breast milk samples (~5-10ml) will be collected from the mother on the day of each session at the WCH and in the evening of the same day (at the participant’s home). A total of 18 breast milk samples will be collected from each participant across the 3 sessions.

**-** Dietary information (24-hour recall) will be completed by women during each session at the WCH

- Maternal weight and height will be measured by study staff before breakfast on the morning of the first session

-Weight and length of infants will be recorded on the morning of each session

-Sociodemographic, basic clinical information and information on habitual diet (food frequency questionnaire) of mothers and infants will be collected by study staff at the first study visit.

### 5.6.4 Summary of Study Timeline

|  |  |  |  |
| --- | --- | --- | --- |
| Time | Activity | Samples collected | Data collected |
| 7:00 | Arrival | Breast milk (~5-10ml) | Dietary information  Maternal weight and height (first session) |
| 7:30 | Breakfast meal | - | - |
| 8:00 | Completion of Breakfast meal | - | - |
|  | Measurement of infants | - | Weight and length |
| 9:00 | 1 hour after breakfast | Breast milk (~5-10ml) | - |
|  | Case Report Form | - | Sociodemographic, clinical and infant information (first session) |
| 10:00 | 2 hours after breakfast | Breast milk (~5-10ml) | - |
| 10:10 | Morning tea |  | Food intake recorded |
| 12:00 | 4 hours after breakfast | Breast milk (~5-10ml) | - |
|  | Lunch |  | Food intake recorded |
| 14:00 | 6 hours after breakfast | Breast milk (~5-10ml) | Meals for the day will be provided. |
| 14:00 | Women returns home | Breast milk (~5-10ml) at ~12 hours after breakfast | Food intake for remainder of the day recorded |
| ~20:00 | ~ 12 hours after breakfast | Breast milk (~5-10ml) |  |

## Sample size

This study will include 25 women/infant pairs. A total of 30 women will be enrolled to allow for a 20% drop out or non-completion of the study per protocol. This will be a pilot proof-of-concept study, and therefore it is not possible to perform a meaningful power calculation.

## Recruitment

Women will be approached by research staff at one of their antenatal appointments or in the postnatal ward after delivery or at postnatal appointments at the Women’s and Children’s Hospital. Women in the postnatal ward will only be approached by a member of our study staff after they have provided consent to a WCHN staff member for us to do so. This consent will be documented on the screening log for the study. Women in the postnatal ward may also be screened and recruited by a WCHN staff member. The study will also be advertised through the South Australian Health and Medical Research Institute (SAHMRI), Child Nutrition Research Centre and SAHMRI Facebook page, Australian Breastfeeding Association and Community centres. The research staff will provide the women with information about the study and, if interested, will screen the women for eligibility. If they are being screened antenatally, eligible women will be asked to sign a Screening Consent Form in which they will provide consent for study staff to access their medical records to identify when they have given birth and to be contacted by study staff. Once consent is given to access their medical records, the research staff will check the system after birth to ensure that women will not be contacted in case of significant issue relating to the birth of their baby.

Women screened antenatally, who have signed a Screening Consent Form, will be contacted by study staff at ~5 weeks post-partum to re-confirm their willingness to take part in the study and re-confirm eligibility. Women who are still interested and eligible will be provided with a copy of the Participant Information and Consent Form and given the opportunity to speak with friends/family and have any questions answered by study staff.

The information sheet will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. The investigator, or research staff/student, will conduct the informed consent discussion and will check that the information provided is understood and answer any questions about the study. Consent will be voluntary and free from coercion. Women willing to participate will sign the written consent form prior to the first session. Following documentation of informed consent, a member of the study research staff will collect baseline data including contact details, demographic data, diet and infants/maternal health-related background data, using the study Case Report Form.

A record of all women screened, eligible and enrolled or not enrolled will be maintained to enable accurate CONSORT reporting. Women may withdraw from the study for any reason at any time. If the women are willing to provide a reason for withdrawal, this will be recorded.

We will aim to advertise study participation by brochures, posters and social media including the CNRC Facebook page and the SAHMRI website. All advertising material will be submitted to HREC for review and approval.

## 5.9 Methods: data collection, management, and analysis

### 5.9.1 Data collection and management

Data will be collected by trained research staff onto paper Case Report Forms. Data entry and management will be coordinated by the research staff/students with cross-checking of at least 10% of the entries.

The Case Report Forms will be stored in a locked office on level 7, SAHMRI at WCHN at the Women’s and Children’s Hospital. Only research staff directly involved in the study will have access to the information. Data will only be released to persons authorised to receive those data.

Original/copies of study documents will be retained on site or in archives. Documents and electronic data will be retained for at least 30 years after study completion in line with the data retention schedules for research involving minors. At the completion of this time documentation will be destroyed using confidential document disposal. The study electronic data will be secure by servers with access only granted to authorised study personnel.

### 5.9.2 Statistical methods

The change in breast milk concentrations of each individual macronutrient and hormone over the 12 hours following the breakfast will be assessed using repeated measures analyses of variance. The area under the concentration curve will be calculated to determine the total change in levels of each hormone/macronutrient across the 12-hour period. The pattern of change and total change in the level of each hormone/macronutrient across the 12-hour period following the 3 different breakfast meals will be assessed using a repeated measures 2-way ANOVA and paired t-test respectively.

**5.10 METHODS: Study oversight and monitoring**

*5.10.1 Steering Committee*

A Steering Committee consisting of the named investigators will review and monitor the progress of the study (recruitment, compliance, data quality and loss to follow-up).

*5.10.2 Adverse Events*

While the risks of participating in this study are very low, we accept the possibility that the mother or infant may have an adverse reaction to one or more of the study breakfasts.

All adverse events will be documented in the Case Report Form and separate Adverse Event form during the course of the study. All adverse events occurring during the period from screening visit/signed informed consent to last visit/study completion will be registered. An independent data monitoring committee will make an assessment of severity, causality, expectedness and seriousness for all adverse events. The Investigator is responsible for reporting adverse events in accordance with current guidelines and regulations to the Human Research Ethics Committee and, if applicable, relevant regulatory authorities.

**ETHICS AND DISSEMINATION**

* 1. **Research ethics approval**

This protocol and the template informed consent forms will be reviewed and approved by the Women’s and Children’s Health Network (WCHN) Human Research Ethics Committee with respect to scientific content and compliance with applicable research and participant regulations.

* 1. **Protocol amendments**

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon by the investigators and WCHN HREC prior to implementation and notified to the health authorities in accordance with local regulations.

Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by the investigators and will be documented. WCHN HREC may be notified of administrative changes at the discretion of CNRC.

* 1. **Confidentiality**

All study-related information will be stored securely at the study site, Women’s and Children’s Hospital. All participant information will be stored in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by ID numbers, study ID and name code only to maintain participant confidentiality. All local databases will be secured with password protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in an area with limited access.

* 1. **Access to data**

All investigators involved in the study will have access to the full data set.

* 1. **Dissemination policy**

The results of this study will be presented at seminars to contribute to publicly available knowledge about the effect of consuming specific dietary factors and short-term changes in breast milk composition. These results, significant or not, will be published to allow non-biased data to be assessed. There is expected to be minimal intervals between the completion of data collection and the release of the study results.

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**APPENDIX 1: Detailed diet information for breakfast meals**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Breakfast meal | Food | Quantity | Energy (kJ) | Fat content (g) | Sugar content (g) |
| High in fat |  | **273.5g** | **2161** | **28.12** | **18.45** |
|  | Coles Apricot Date & Almond Muesli | 30g | 524 | 4.66 | 5.17 |
|  | Devondale Full Cream Milk | 100mL | 289 | 3.50 | 6.18 |
|  | Jalna Greek Yogurt Natural | 90g | 530 | 9.09 | 6.21 |
|  | Helga’s Continental Bakehouse Soy & Linseed | 42.5g | 575 | 4.36 | 0.89 |
|  | Western Star Butter Original | 8g | 242 | 6.50 | 0.00 |
|  |  |  |  |  |  |
| High in sugar |  |  | **2100** | **4.95** | **56.60** |
|  | Sanitarium Weet Bix Fruity | 30g | 459 | 0.27 | 6.90 |
|  | Devondale Semi-Skim Milk | 100mL | 199 | 1.25 | 5.20 |
|  | Coles Strawberry Lite Yogurt | 100g | 368 | 0.90 | 14.60 |
|  | Tip Top Raisin Toast | 65g | 748 | 2.54 | 9.75 |
|  | Coles Raspberry Jam | 15g | 165 | 0.00 | 10.08 |
|  | Honey | 1 teaspoon | 94 | 0.00 | 5.87 |
|  | Sugar, white, regular | 1 teaspoon | 67 | 0.00 | 4.20 |
|  |  |  |  |  |  |
| Breakfast meal | **Food** | **Quantity** | **Energy (kJ)** | **Fat content (g)** | **Sugar content (g)** |
| Control |  |  | **2132** | **11.78** | **25.15** |
|  | Be Natural 5 Whole Grain Flakes | 45g | 670 | 1.85 | 5.36 |
|  | Devondale Skim Milk | 200mL | 397 | 2.5 | 10.4 |
|  | YoPRO high protein vanilla yoghurt | 160g | 397 | 0.3 | 6.1 |
|  | Golden Crumpets with wholemeal | 1 round crumpet | 349 | 0.46 | 0.63 |
|  | Strawberry, fresh | 70g | 76 | 0.14 | 2.66 |
|  | Devondale Australian Butter | 8g | 243 | 6.54 | 0 |