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Clinical Application of the Food Insulin Index to Diabetes Mellitus

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B.Nutr&Diet (Hons), GradCertDiabEd (Dist)

*Submitted in total fulfillment of the requirements of the
degree of Doctor of Philosophy*

5 September 2014



THE UNIVERSITY OF
SYDNEY

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Declaration

The work presented in this thesis, to the best of my knowledge, is original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part for a degree at this or any other institution.

The contribution of other people to the research reported in the thesis is duly acknowledged in the following section. The research protocols were approved by the University of Sydney Human Research Ethics Committee. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliographies and appendices.

Assistance with the practical work for the assessment of the Food Insulin Index values of some of the foods presented in Chapter 3 was provided by Masters of Nutrition and Dietetic students, Sally Lane and Aleksandra Grasar.

Signed:

.....

Kirstine Bell

5 September 2014

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Text Abbreviations

AA	Amino Acid
ADA	American Diabetes Association
AMP	Adenosine Monophosphate
ASP	Aspartate
ATP	Adenosine Triphosphate
AvCHO	Available Carbohydrate
BCAA	Branch Chain Amino Acid
BG	Blood Glucose
BGL	Blood Glucose Level
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CC	Carbohydrate Counting
CDE	Credentialed Diabetes Educator
CGMS	Continuous Glucose Monitoring System
CHD	Coronary Heart Disease
CHO	Carbohydrate
C-Peptide	Connection Peptide

CRP	C-Reactive Protein
CSII	Continuous Subcutaneous Insulin Infusion
CU	Carbohydrate Unit
CVD	Cardiovascular Disease
DAFNE	Dose Adjustment For Normal Eating
DCCT	Diabetes Control and Complications Trial
DTTP	Diabetes Teaching and Treatment Program
FA	Fatty Acids
FFA	Free Fatty Acids
FID	Food Insulin Demand
FII	Food Insulin Index
FPU	Fat-Protein Unit
GABA	Gamma-Aminobutyric Acid
GDH	Glutamate Dehydrogenase
GI	Glycaemic Index
GIP	Glucose-Dependent Insulinotropic Polypeptide
GK	Glucokinase
GL	Glycaemic Load

GLP-1	Glucose-Like Peptide-1
Glyc-3-P	Glycerol-3-Phosphate
GS	Glucose Score
GSIS	Glucose-Stimulated Insulin Secretion
HbA1c	Haemoglobin A1c
HDL	High Density Lipoprotein
iAUC	Incremental Area Under the Curve
ICR	Insulin: Carbohydrate Ratio
IFR	Insulin: FID Ratio
LC-CoA	Long-Chain Acyl CoA
LDL	Low Density Lipoprotein
MAGE	Mean Amplitude Glycaemic Excursion
MAIE	Mean Amplitude Insulinaemic Excursion
MCF	Metabolic Coupling Factor
mCAT	Messenger Catatonic Amino acid Transporter
MDH	Malate Dehydrogenase
mRNA	Messenger RNA (Ribonucleic Acid)
mTOR	Mammalian Target Of Rapamycin

MUFA	Monounsaturated Fat
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
OOA	Oxaloacetate
PC	Pyruvate Carboxylase
PD	Pyruvate Dehydrogenase
PDG	Phosphate-Dependent Glutaminase
PKC	Protein Kinase C
PUFA	Polyunsaturated Fat
RCT	Randomised Controlled Trial
SD	Standard Deviation
SEM	Standard Error of the Mean
SMBG	Self-Monitoring of Blood Glucose
TCA	Tricarboxylic Acid
UCP	Uncoupling Protein
WPTS	Warsaw Pump Therapy School

Publications resulting from this research

Peer-reviewed papers

1. **Bell K**, Barclay AW, Petocz P, Colagiuri S, Brand-Miller JC. The efficacy of carbohydrate counting in type 1 diabetes: a systematic review and meta-analysis. *Lancet Diabetes & Endocrinology* 2014; 2(2): 133-140.
2. **Bell K**, Gray R, Munns D, Petocz P, Howard G, Colagiuri S, Brand-Miller JC. Estimating insulin demand for protein-containing foods using the Food Insulin Index vs carbohydrate counting. *European Journal of Clinical Nutrition*. 2014; 68: 1055-1059.

Manuscripts in preparation

3. **Bell K**, Petocz P, Colagiuri S, Brand-Miller JC. Testing of foods & beverages for their Food Insulin Index and the systematic evaluation of the associations between postprandial glycaemia and insulinaemia and nutritional content.
4. **Bell K**, Gray R, Munns D, Petocz P, Howard G, Colagiuri S, Brand-Miller JC. Clinical application of the Food Insulin Index in the diet of adults with type 1 diabetes: The FOODII Study
5. **Bell K**, Bao J, Petocz P, Colagiuri S, Brand-Miller JC. Effect of diets of varying Food Insulin Index on day-long glucose and insulin profiles in adults with type 2 diabetes

Published abstracts

1. Bao J, Atkinson F, **Bell K**, Willett WC, Brand-Miller JC. Development of a Food Insulin Index (FII) [Abstract] *Nutrition & Dietetics* 2012; 69(S1): 2.

2. **Bell K.** Carbohydrate counting in type 1 diabetes. *Diabetes Management Journal* 2013; 42:16-17
3. **Bell K, Barclay AW, Petocz P, Colagiuri S, Brand-Miller JC.** The efficacy of carbohydrate counting in type 1 diabetes: a systematic review and meta-analysis [Abstract] *Diabetes* 2013; 62(S1):A42
4. **Bell K, Gray R, Munns D, Petocz P, Howard G, Colagiuri S, Brand-Miller JC.** Estimating Insulin Demand for Protein-Containing Foods Using the Food Insulin Index vs Carbohydrate Counting [Abstract] *Diabetes* 2013; 62(S1):A97
5. **Bell K, Gray R, Munns D, Petocz P, Howard G, Colagiuri S, Brand-Miller JC.** Food Insulin Index (FII) vs. Traditional Carbohydrate Counting for Glycemic Control in Adults with Type 1 Diabetes: A 3-Month Pilot Study [Abstract]. In Press.

Other publications

1. **Bell, K.** Carbohydrate counting in type 1 diabetes. *Diabetes Management Journal* 2013; 42:16-17

Presentations resulting from this research

Invited speaker

1. **Bell, K.** Estimating Mealtime Insulin Dose: The Food Insulin Index. Baker IDI and Diabetes Australia – Victoria Health Professionals Symposium: The “E’s” of Diabetes. Melbourne, Australia. May, 2012.
2. **Bell, K.** The Food Insulin Index. Dietitians Association of Australia NSW Branch Diabetes Interest Group Meeting. Sydney, Australia. February, 2013.
3. **Bell, K.** The Food Insulin Index - a new method for dosing insulin? The Dietitians Association of Australia (DAA) Queensland Branch Executive & Diabetes Interest Group: If you aren’t doing diabetes – what are you doing? Brisbane, Australia. March, 2013.
4. **Bell, K.** Dietitians Association of Australia (DAA) Victorian Diabetes and Heart Health Event: The Now & the Future of Diabetes & Heart Health. Melbourne, Australia. April, 2013.
5. **Bell, K.** Food Insulin Index: Improving Estimation of Mealtime Insulin Dose in Adults with Type 1 Diabetes Mellitus as part of the Symposium: Mealtime Insulin Dosing–It’s More Than Counting Carbohydrates. American Diabetes Association 73rd Scientific Sessions Meeting. Chicago, USA. June, 2013.
6. **Bell, K.** New Methods for Dosing Insulin in Type 1 Diabetes. Australia Diabetes Society & Australian Diabetes Educators Association’s Annual Scientific Meeting. Sydney, Australia. August, 2013.

7. **Bell, K.** Improving Mealtime Insulin Dosing with the Food Insulin Index. Royal North Shore Hospital: Endocrine, Metabolism & Diabetes Friday Lunchtime Research Meeting. Sydney, Australia. October 2013.
8. **Bell, K.** Improving Mealtime Insulin Dosing with the Food Insulin Index. Westmead Hospital: Dietitians Continuing Education Sessions. Sydney, Australia. May, 2014.
9. **Bell, K.** Improving Mealtime Insulin Dosing with the Food Insulin Index. Westmead Hospital: Grand Rounds. Sydney, Australia. May, 2014.
10. **Bell, K.** Improving Mealtime Insulin Dosing with the Food Insulin Index. Charles H. Best 2014 Annual Continuing Education Conference: State of the Art Management of the Adult with Type 1 Diabetes. Ajax, Canada. May, 2014.

Presenter

1. **Bell K.** Food Insulin Index (FII) vs. Traditional Carbohydrate Counting for Glycemic Control in Adults with Type 1 Diabetes: A 3-Month Pilot Study. The American Diabetes Association's 74th Scientific Sessions Meeting. San Francisco, USA. June, 2014.
2. **Bell, K.** Development of a Food Insulin Index (FII). The 16th International Congress of Dietetics. Sydney, Australia. September, 2012.
3. **Bell, K.** The efficacy of carbohydrate counting in type 1 diabetes: a systematic review and meta-analysis. The American Diabetes Association's 73rd Scientific Sessions Meeting. Chicago, USA. June, 2013.

Poster

1. **Bell, K.** Effect of diets of varying food insulin index (FII) on day-long glucose and insulin profiles in adults with type 2 diabetes. The World Diabetes Congress, Melbourne, Australia. December 2013.

Abstract

Type 1 diabetes is an autoimmune disorder characterised by hyperglycaemia resulting from β -cell destruction and absolute deficiency of endogenous insulin. Current medical management revolves around exogenous insulin therapy to restore blood glucose levels to the optimal range. Despite concerted efforts on the part of clinicians, researchers and patients, euglycaemia remains difficult to achieve in day-to-day practice particularly in response to meals high in protein and/or fat. Given the risk of life-threatening acute and chronic diabetic complications resulting from inadequate glycaemic control, improving the insulin dose algorithm warrants further research.

The Food Insulin Index (FII) has been proposed as an alternative strategy for mealtime insulin dosing in type 1 diabetes. The FII is a novel system of ranking foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food. Using food energy rather than carbohydrate as the constant allows all foods to be included (not just those with sufficient carbohydrate), and thus *all* dietary components and their metabolic interactions can be considered, allowing a more holistic approach to determining insulin demand. The goal of the research described in this thesis was to deepen our knowledge of the relationship between foods and normal physiological insulin demand and explore the clinical application of this novel 'insulin index' of foods to diabetes management. We hypothesised that there was only limited evidence to support carbohydrate counting as the primary dietary therapy in type 1 diabetes and that the use of the FII would improve glycaemic control beyond that of carbohydrate counting. We also

hypothesised that the FII could also improve metabolic control in type 2 diabetes by reducing insulinaemia.

Carbohydrate counting is currently considered the gold standard method for determining prandial insulin dose in type 1 diabetes mainly on the basis of narrative review. We therefore conducted a systematic review and meta-analysis of studies assessing the efficacy of carbohydrate counting on glycaemic control in adults and children with type 1 diabetes (Chapter 2). Only 7 studies that met the inclusion criteria and meta-analysis revealed there was no significant improvement in glycosylated haemoglobin A1c (HbA1c) with carbohydrate counting over general dietary advice and/or usual care (-0.35%, $p = 0.096$). This study highlighted the need for research into alternative strategies to improve the accuracy of the mealtime insulin dose in type 1 diabetes.

Chapter 3 presents the results of testing 26 new foods for their FII value in healthy subjects and exploratory analysis of the complete database of 147 foods. Linear regression analysis of nutrients vs FII indicated that postprandial insulinaemia is not the response to a single nutrient (carbohydrate) but rather the sum total effect of metabolic *interactions* among different nutrients within foods. Although a predictive equation based on the GL and protein content could be generated, this explained only 57% of observed insulin responses in healthy individuals. Because the FII cannot be accurately calculated based on the known nutrient content of the food, it is best determined through *in vivo* testing.

The FII reveals a notable insulin demand for foods high in protein and fat, nutrients that would normally be disregarded for mealtime insulin dosing with traditional carbohydrate counting. As it is conceivable that using the FII to estimate insulin

doses for foods rich in protein and fat might result in hypoglycaemia, the next stage of this research assessed the safety of using the FII for these foods as evening meals. Six high protein foods with little or no carbohydrate were studied (Chapter 4). Compared with carbohydrate counting, the FII algorithm was able to reduce mean blood glucose level (5.7 ± 0.2 mmol/L vs 6.5 ± 0.2 mmol/L, $p = 0.003$), while the rate of hypoglycaemia was similar in both conditions (48% vs 33% for FII vs carbohydrate counting respectively, $p = 0.155$). Thus, even in this ‘worst case’ scenario, the FII appears to be no less reliable than carbohydrate counting for preventing hypoglycaemia.

Although the FII had been trialled in the research setting, it remained to be seen whether the FII was feasible in practice and could be translated into clinically meaningful improvements in long-term glycaemic control. In the first randomised, controlled trial of the real-world application of the FII, 26 adults with type 1 diabetes counted FID units or carbohydrate grams and calculated their prandial insulin doses using an individualised insulin ratio (Chapter 5). The results indicated that FID counting was at least as good as carbohydrate counting for glycaemic control (FII: $-0.1 \pm 0.1\%$ vs CC: $-0.3 \pm 0.2\%$, $p = 0.855$), with a trend towards reduced risk of hypoglycaemia in the FID counters after 12 weeks (-43% , $p = 0.057$), inferring improved glycaemic stability.

The potential of the FII to improve glycaemic control is not just limited to type 1 diabetes. Chronic hyperinsulinaemia may exacerbate insulin resistance and β -cell failure in type 2 diabetes. The FII offers an alternate dietary approach to reducing postprandial insulin demand and thus hyperinsulinaemia. The final study therefore explored this potential by comparing the day-long plasma glucose and insulin

responses of 10 adults with non-insulin treated type 2 diabetes to a low-FII vs a high-FII diet (Chapter 6). As hypothesised, the low FII diet produced a significantly lower day-long insulin response compared to a high FII diet (iAUC_{insulin} 18,740 ± 3,100 pmol/L vs 11,000 ± 1,810 pmol/L, p = 0.018), even when matched for macronutrients, fibre and GI. Hence, FII represents a promising dietary strategy for reducing postprandial hyperinsulinaemia in type 2 diabetes, thereby reducing insulin resistance and preserving β -cell function.

Collectively, these studies offer exciting insights into the relationship between food and normal physiological insulin secretion and the potential of the FII for optimising glycaemic control and managing both type 1 and type 2 diabetes. Until a cure for diabetes can be found, the potential for clinically significant enhancements in overall glucose control and reduced glycaemic variability offer people with diabetes greater wellbeing through a reduced burden of disease and decreased risk of long-term diabetes complications.

CHAPTER 1

Introduction and
literature review

1.1 Introduction

Type 1 diabetes is an autoimmune disorder characterised by hyperglycaemia resulting in an absolute endogenous insulin deficiency. This life-threatening disorder affects almost 500,000 children under 15 years of age worldwide, and is estimated to be increasing by around 3% per year. Australia ranks in the top 10 countries worldwide with the highest rates of type 1 diabetes¹. Current medical management revolves around exogenous insulin therapy to restore blood glucose levels to within an optimal range. At present there is no cure for type 1 diabetes, therefore effective strategies to assist in the achievement and maintenance of normoglycaemia are required to promote the acute and long-term health and wellbeing of individuals with type 1 diabetes.

Carbohydrate counting has long been considered the cornerstone of intensive insulin therapy, with bolus insulin doses matched to the total carbohydrate content of the meal². This practice is based on the premise that carbohydrate is the predominant macronutrient contributing to the rise in post-prandial glycaemia, however the underlying theoretical basis and its practical utility have since been questioned³⁻⁷. Current international recommendations supporting the use of carbohydrate counting in practice are based simply on narrative review and grading of the limited available evidence^{2,6}.

Despite significant advancements in insulin therapy, optimum postprandial glycaemic control remains difficult to achieve. Even patients within target glycated haemoglobin A1c (HbA1c) levels continue to experience unanticipated hyper- and hypoglycaemia, particularly in response to meals high in protein and/or fat^{3,8,9}. Given the risk of developing life-threatening acute and chronic diabetic complications, improving the insulin dose algorithm presents a significant clinical issue.

Numerous studies in healthy subjects have demonstrated that the same amount of carbohydrate from different food sources produces wide variations in the normal blood glucose and insulin responses^{3, 10-12}. In addition, *in vitro* and *in vivo* studies have demonstrated the role of protein and fat in addition to carbohydrate on normal physiological insulin secretion¹³⁻¹⁵. A more comprehensive understanding of the relationship between dietary factors and physiological insulin secretion evoked by foods is, therefore, of clinical and practical importance in the management of type 1 diabetes.

To address this gap in the evidence and thus clinical practice, a Food Insulin Index (FII) has been proposed. The FII is a novel algorithm of ranking foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food¹⁶. Using food energy as the constant allows all foods to be included, not just those with sufficient carbohydrate content, and thus all dietary components and their metabolic interactions can be considered, allowing a more holistic approach to determining insulin demand. Previous research in healthy people has demonstrated that the FII algorithm is a more accurate predictor of observed insulin responses to composite meals than carbohydrate content¹⁷. Furthermore a preliminary clinical trial in adults with type 1 diabetes yielded promising results, showing the FII algorithm was associated with improved postprandial glycaemia without increased risk of hypoglycaemia compared to carbohydrate counting following a mixed meal¹⁸. The goal of the research described in this thesis therefore, was to deepen our knowledge of the relationship between foods and normal physiological insulin demand and explore the clinical application of this novel 'insulin index' of foods to diabetes management. We hypothesised that there was only limited evidence to support carbohydrate counting as the primary dietary therapy in type 1 diabetes and that the use of the FII would improve

glycaemic control beyond that of carbohydrate counting. We also hypothesised that the FII could also improve metabolic control in type 2 diabetes by reducing insulinaemia.

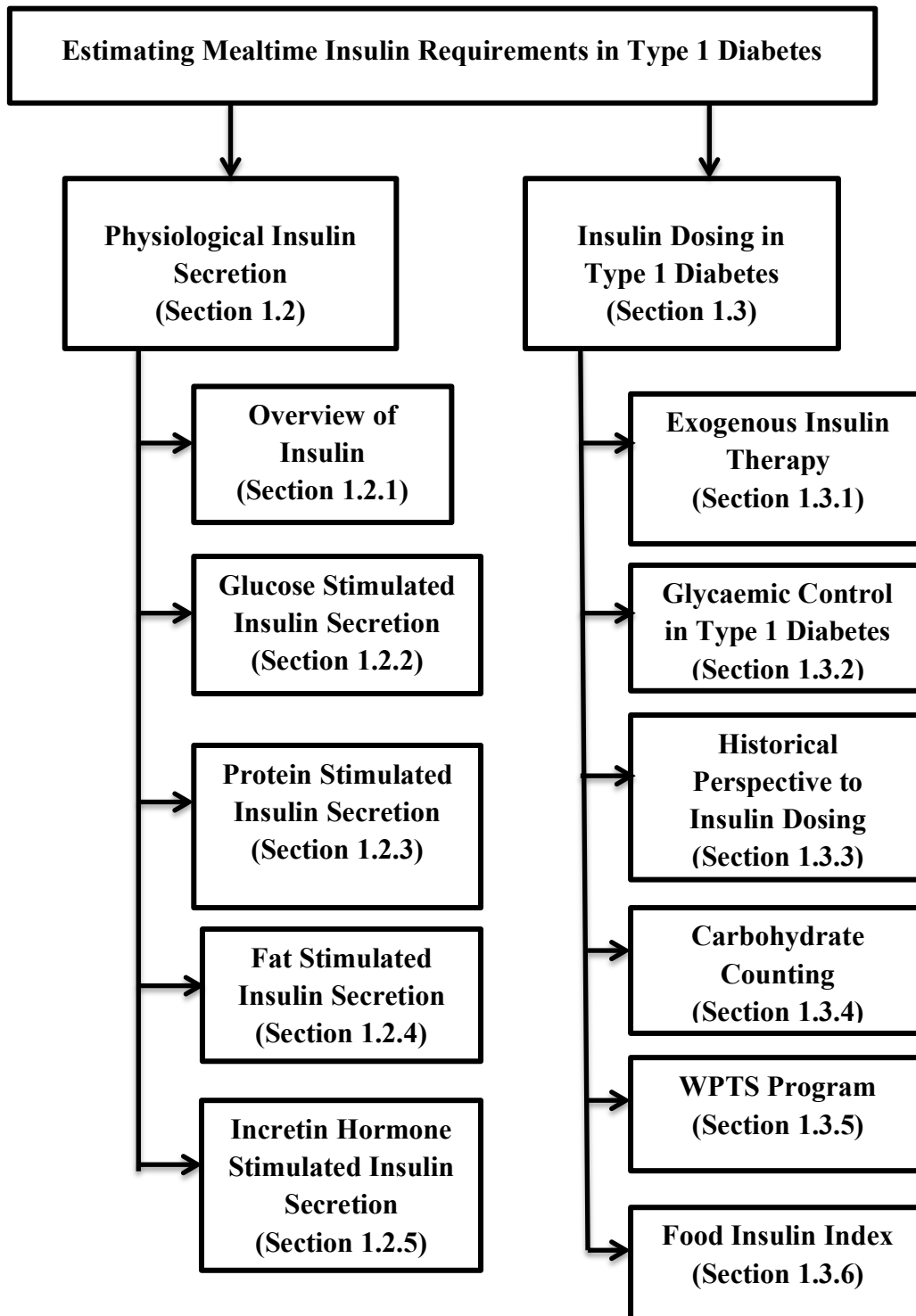


Figure 1.1: Framework of literature review

1.2 Physiological insulin secretion

1.2.1 Overview of insulin

1.2.1.1 Physiological insulin function

Insulin is a polypeptide hormone that plays a central role in the regulation of glucose homeostasis. It is the most potent anabolic hormone and its principal action is to:

- Facilitate the entry of glucose and amino acids into the cells
- Suppress the release of glucose from the liver
- Promote the synthesis and storage of glycogen and fats

1.2.1.2 Structure and biosynthesis of insulin

Insulin is synthesised in the β -cells of the Islets of Langerhans in the pancreas following the systematic production and processing of its biologically inactive precursors.

Insulin is composed of two peptide chains, the A and B chains, connected by two disulphide bonds, with an additional disulphide bond in the A chain (**FIG 1.2.1.1**)¹⁹. In humans, the A chain consists of 21 amino acids while the B chain is made up of 30 amino acids.

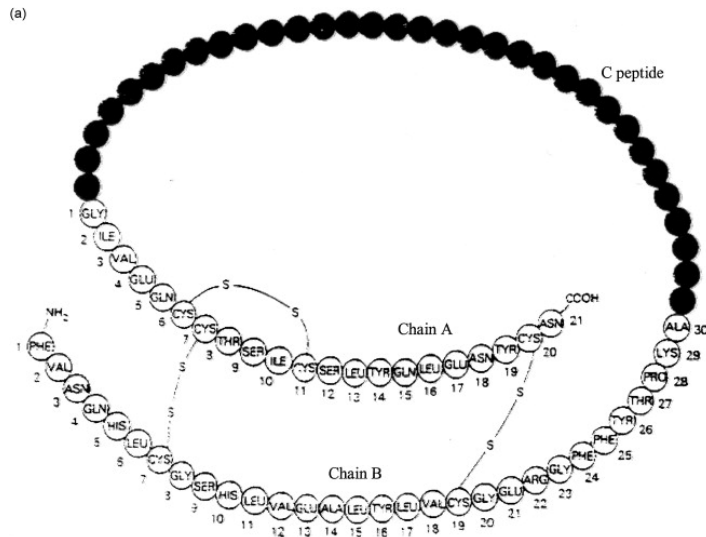


Figure 1.2.1.1: Chemical structure of Insulin: Two peptide chain (A and B chains) connected by two disulphide bonds, with an additional disulphide bond in the A chain. Insulin is synthesised from proinsulin (pictured) by the removal of the C-peptide chain¹⁹.

Insulin biosynthesis begins with the translation of insulin mRNA to produce preproinsulin in the β -cell. This single-chain molecule has a 24 amino acid signal peptide attached to the N-terminus (**FIG 1.2.1.2A**²⁰). The signal peptide facilitates the translocation of the preproinsulin from the cytosol across the rough endoplasmic reticulum membrane and into the cell lumen. During this translocation, preproinsulin is converted into the prohormone proinsulin by signal peptidases, which remove the signal peptide (**FIG 1.2.1.2B**²⁰). Within the endoplasmic reticulum, several specific endopeptidases excise the 31 amino acid C-peptide chain from proinsulin to derive the bioactive insulin molecule (**FIG 1.2.1.2C**²⁰). The insulin is then stored in the β -cell until the cell is stimulated to release the insulin into circulation.

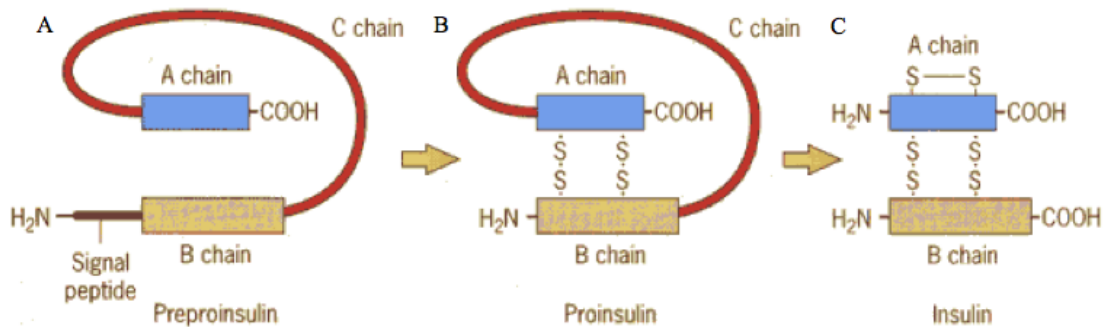


Figure 1.2.1.2: Structure of A) Preproinsulin, B) Proinsulin and C) Insulin, showing the signal peptide, A and B chains linked by disulphide bonds and C-peptide and the processing from the biologically inactive preproinsulin into the bioactive insulin hormone²⁰.

Under normal circumstances, the β -cell maintains a readily available pool of insulin that can be rapidly secreted in response to a stimulus, such as an increase in blood glucose. Any insulin release is compensated for by a corresponding increase in insulin biosynthesis, so that β -cell insulin stores are constantly maintained.

1.2.1.3 *Insulin secretion*

Insulin is a potent regulator of metabolism and the β -cell is exquisitely designed to detect and respond to the ingestion of carbohydrate, protein, fat, gut hormones, neural stimulation and pharmacological agents to stimulate and/or amplify insulin secretion.

1.2.2 Glucose-stimulated insulin secretion

1.2.2.1 Introduction

Elevation of the blood glucose level is the most potent stimuli for insulin release from the β -cell in the pancreas. In 1981, Slama et al. showed there was a highly significant correlation between the amounts of carbohydrate consumed, as sugar (dextrose) or as part of mixed meals, and the total amount of insulin needed to restore blood glucose levels to baseline levels in type 1 diabetes using an artificial pancreas²¹. Further work in this field confirmed the relationship between carbohydrate intake and insulin requirement^{3, 4, 22, 23}. However, these studies failed to account for other factors affecting normal insulin secretion in healthy subjects, as discussed in the following sections.

1.2.2.2 Mechanism

In the presence of elevated blood glucose concentrations, glucose is transported into the cytosol of β -cell in the pancreas by the GLUT-2 transporter. This process occurs independently of insulin. Once inside the β -cell, glucose is phosphorylated into glucose-6-phosphate by glucokinase (GK). As this is the rate-limiting step, it effectively traps the glucose inside the β -cell. Glucose-6-phosphate undergoes glycolysis, producing pyruvate in the cytosol. Pyruvate enters the mitochondria as acetyl-CoA and continues through the citric acid cycle and electron transport system, producing multiple high-energy ATP molecules. Each glucose molecule oxidised produces 38 ATP molecules through aerobic metabolism. The additional ATP molecules produced increases the ATP:ADP ratio. The ATP molecules bind to the 'ATP-gated K^+ channel', closing the channel and thus preventing K^+ ions from 'leaking' out. Retention of the K^+ ions depolarises the cell from -70 mV, which opens the

voltage-sensitive Ca^{2+} channels when the membrane potential reaches -30 mV, and allows the Ca^{2+} enter the β -cell. The increased concentration of Ca^{2+} ions acts as an intracellular signal triggering the exocytosis of insulin from the β -cell and into the blood (**FIG 1.2.2.1**).

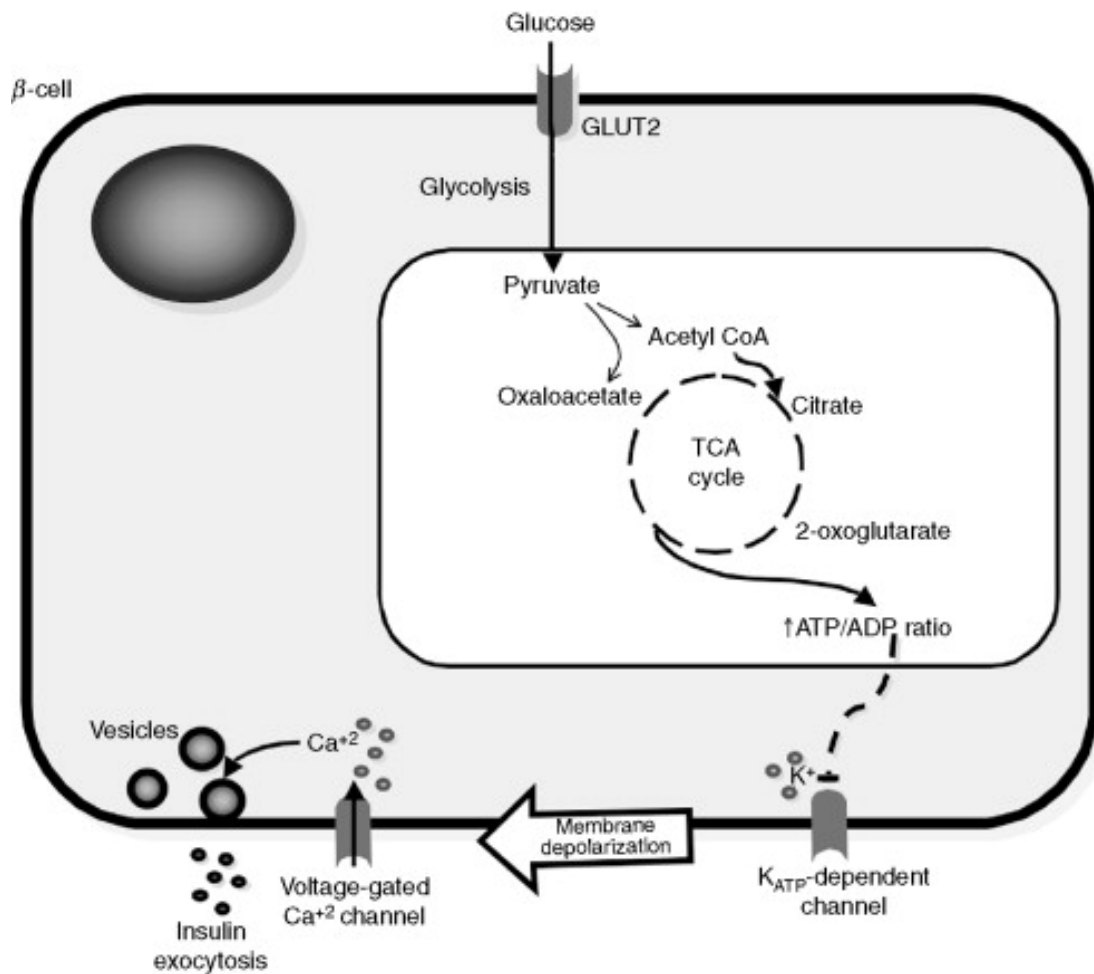


Figure 1.2.2.1: Schematic diagram of glucose-stimulated insulin secretion²⁴

1.2.2.3 Effect of different carbohydrate sources

The type of carbohydrate consumed is also an important factor in determining the insulinaemic response. Available carbohydrate alone has been shown to account for just 49%

of the blood insulin response to single foods and there is only a marginal correlation between available carbohydrate and insulin response for mixed meals ($r = 0.53$, $p = 0.06$)¹⁶. However when both the amount and type of carbohydrate (as quantified by the Glycaemic Index (GI), a physiological measure of the glycaemic response to carbohydrate-containing foods) is taken into consideration, the two factors explain 59% of the variation in insulin response to single foods and 46% of the variation to mixed meals¹⁶.

1.2.3 Protein-stimulated insulin secretion

1.2.3.1 Introduction

Although protein has a negligible effect on blood glucose levels in healthy subjects, it has shown to be a potent insulin secretagogue. Physiologically, insulin is needed to promote the uptake of amino acids and synthesis of new proteins.

Dietary proteins are able to directly stimulate insulin secretion, as amino acid metabolism in the β -cell shares common pathways with glucose metabolism, which leads to the exocytosis of the insulin vesicles from the β -cell, ie insulin secretion¹⁴. The specific mechanisms of protein-stimulated insulin secretion will be discussed in detail later in this section.

Dietary protein is digested into small peptides and free amino acids by proteases in the stomach and small intestine, which can then be easily transported across the intestinal wall by a wide variety of transporters²⁵. Compared with carbohydrate metabolism, this is a relatively slow process, requiring around 8 h, although the majority of protein is digested and absorbed within 4 h²⁶. The gut mucosal cells catabolise aspartate, glutamate and glutamine for fuel, while the remaining amino acids are transported to the liver through the portal vein. Once in the liver, the nonessential amino acids are largely deaminated, with the nitrogen excreted and

the carbon skeleton used for glucose synthesis. The essential amino acids however, are passed into general circulation where they can be used for new protein synthesis or skeletal muscle fuel if required. It is these circulating amino acids, which stimulate insulin secretion²⁷.

1.2.3.1.1 Healthy subjects

In isolation, protein is considered a relatively weak stimulus for insulin secretion compared to glucose, however when combined with carbohydrate, the resulting insulin response is significantly increased above that of glucose alone²⁷⁻³⁰.

In healthy subjects, ingestion of 50 g of protein as lean beef stimulated 72% less insulin than that following the ingestion of 50 g of glucose²⁹. When both glucose and protein was consumed, the insulin response was 127% greater than that of glucose alone, indicating an additive effect between protein and glucose.

Rabinowitz et al. also found there was only a slight increase in plasma insulin following the ingestion of protein alone, however when both protein and glucose were combined, the resulting insulin dose was twice that of glucose alone and four times that of protein alone³¹.

These findings are similar to that observed by Berger and Vongaraya, who found that the insulin response to 50-100 g of protein was 21% of that following the ingestion of 100 g glucose²⁸. This result was consistent regardless of variations in the portion size of protein and whether the protein source was casein or gelatin.

A more recent study found that insulin secretion was increased by 166% when carbohydrate and protein were combined, compared with that of glucose alone³². Nilsson et al also found

some protein sources alone (including milk, cheese, whey and cod) could increase insulin secretion by 124-190% above that of the response to carbohydrate (white bread)³³.

There is further evidence to suggest the dose-response to protein is not linear. When 16 g of protein was added to a liquid test meal containing 58 g of sugars and 0 g of protein, the insulin response doubled³⁴. However, when the protein was increased to 50 g, there was no further increase in the insulin response. In fact there were no significant differences in the insulin response whether the protein load was 16, 25, 34 or 50 g.

1.2.3.1.2 Type 2 diabetes

The insulin response to protein in adults with type 2 diabetes is remarkably higher than that of healthy subjects. When glucose was combined with protein, the resulting insulin response was nearly double that of the response to glucose alone and about 4-fold higher than the insulin response observed in healthy subjects³⁵. Likewise, the insulin response to the ingestion of protein alone was 80% of that following the ingestion of glucose alone in those with type 2 diabetes, compared with just 21% in healthy subjects²⁸. Manders et al. also found there was a significant increase in the plasma insulin response in subjects with type 2 diabetes after the consumption of carbohydrate with protein, compared with healthy subjects³⁶. The response to carbohydrate with protein was 66% higher than the response to carbohydrate alone in healthy subjects but 141% higher in those with type 2 diabetes.

When seven subjects with type 2 diabetes were given 50 g of glucose, 50 g of protein and 50 g of carbohydrate with 50 g of protein combined on separate occasions, there were similar plasma insulin responses to both glucose and protein when each was given alone, however the response was 2.5 times greater for the combination than the response to either macronutrient

individually and 30% greater than the sum of the individual responses to glucose and protein³⁵. These results suggest that although the insulin response to carbohydrate and protein combined is additive of the responses to the individual macronutrients in healthy subjects, there is a synergistic relationship in type 2 diabetes, increasing the insulin response above and beyond that of the individual responses. Furthermore, similar to when protein was given alone, the plasma insulin response to glucose and protein combined had not returned to the fasting level after 5 h, indicating the final response may have been even greater than that observed within the time constraints of the experiment.

1.2.3.1.3 Type 1 diabetes

The effect of protein on blood glucose levels is dependent on having sufficient amounts of insulin. In the absence of sufficient exogenous insulin in people with type 1 diabetes, it has been shown that protein can raise the blood glucose level.

Smart and colleagues demonstrated that increasing the protein content of the meal independent of the other macronutrients increases the postprandial blood glucose response³⁷. In this study, the protein content of the meal was raised from 5 g to 40 g while the other macronutrients remained constant and the insulin dose was determined by the carbohydrate content of the meal (ie the same insulin dose was given under both conditions as the carbohydrate content did not vary). The mean postprandial glucose excursions after the high protein meal were 2.6 mmol/L higher than the glucose excursions after the low fat meal between 210 to 300 mins (-0.3 mmol/L vs. -2.9 mmol/L; $p = 0.01$), demonstrating that protein in the absence of sufficient insulin raises blood glucose levels and thus there is an insulin requirement for protein in type 1 diabetes.

1.2.3.2 Effect of different protein sources

Although almost all protein sources are capable of stimulating insulin secretion in the absence of carbohydrate, the potency of protein on insulin secretion appears to depend on the quality of the protein consumed, with the literature revealing that different protein sources have different effects on insulin secretion. Pal and Ellis compared four different protein sources in lean, healthy men and found that while each produced a significant insulin response, whey was the most potent secretagogue, followed by fish, turkey and then egg³⁸. Nilsson et al also found whey to be the most potent of all protein sources tested, with a plasma insulin response 90% higher than the response to white bread in healthy subjects³³. The insulinotropic properties of whey may be due, at least in part, to that fact that it is rapidly digested and thus readily bioavailable. Milk and cheese also produced insulin responses 24 and 25% greater than the response to white bread respectively, however the differences were not statistically significant³³. In contrast to the other test foods, the insulin response to cod fish was 11% lower than that of white bread.

In adults with type 2 diabetes, the insulin response to each protein food tested (cottage cheese, gelatin, beef, turkey, fish, soy and egg whites) when combined with glucose was more than two-fold greater than the response to glucose alone, even though the amount of protein was only half that of glucose on a gram-for-gram basis³⁹. The relative area under the curve was greatest for glucose + cottage cheese, with a response 360% greater than that of glucose alone. The lowest response was for glucose + egg whites, however this was still significantly higher than that for glucose alone, producing a plasma insulin response 190% greater than glucose alone.

These relative differences in the insulinotropic properties of different protein sources are similar to that reported by Bao et al.¹⁶ On an isoenergetic basis, dairy products such as skim

milk, cheese and yoghurt produced an insulin response more than two-fold greater than other protein sources such as roast chicken, beef steak and walnuts. Interestingly they also reported differences within protein sources, for example skim milk produced an insulin response more than twice that of full-cream milk. Some protein sources also produced an insulin response comparable to that of carbohydrate sources, for example beef steak produced a similar response to that of grain bread.

1.2.3.3 Mechanism of individual amino acids

The differences in the reported insulinotropic properties of different protein sources may be attributable to the variations in the amino acid composition of the proteins employed. Only certain amino acids can acutely and chronically regulate insulin secretion and the amino acids vary in their mode of action and the magnitude of their insulinotropic effect¹⁴. In descending order, arginine, lysine, leucine and phenylalanine are the most potent insulin secretagogues, however all essential amino acids, with the exception of histidine, stimulate some insulin release⁴⁰. Intravenous administration of a 30 g mixture of all 10 essential amino acids and of certain individual amino acids produces larger increases in plasma insulin than does the infusion of 30 g of glucose. A mixture of the 10 essential amino acids however, is more potent than any single amino acid suggesting there is a synergistic effect among amino acids⁴⁰. It has been reported that individual amino acids can induce insulin secretion only at high concentrations or in combination rather than physiological concentrations in vitro^{14, 15}.

Different amino acids employ different mechanisms to stimulate insulin secretion directly. There are three primary mechanisms within the β -cell: 1) metabolism with generation of ATP (L-alanine, L-proline, L-leucine, L-glutamine), 2) co-transport with sodium resulting in

membrane depolarisation (L-alanine, L-proline) and 3) uptake of cationic amino acids (L-arginine, L-lysine) and membrane depolarisation⁴¹ (FIG 1.2.3.1)⁴¹.

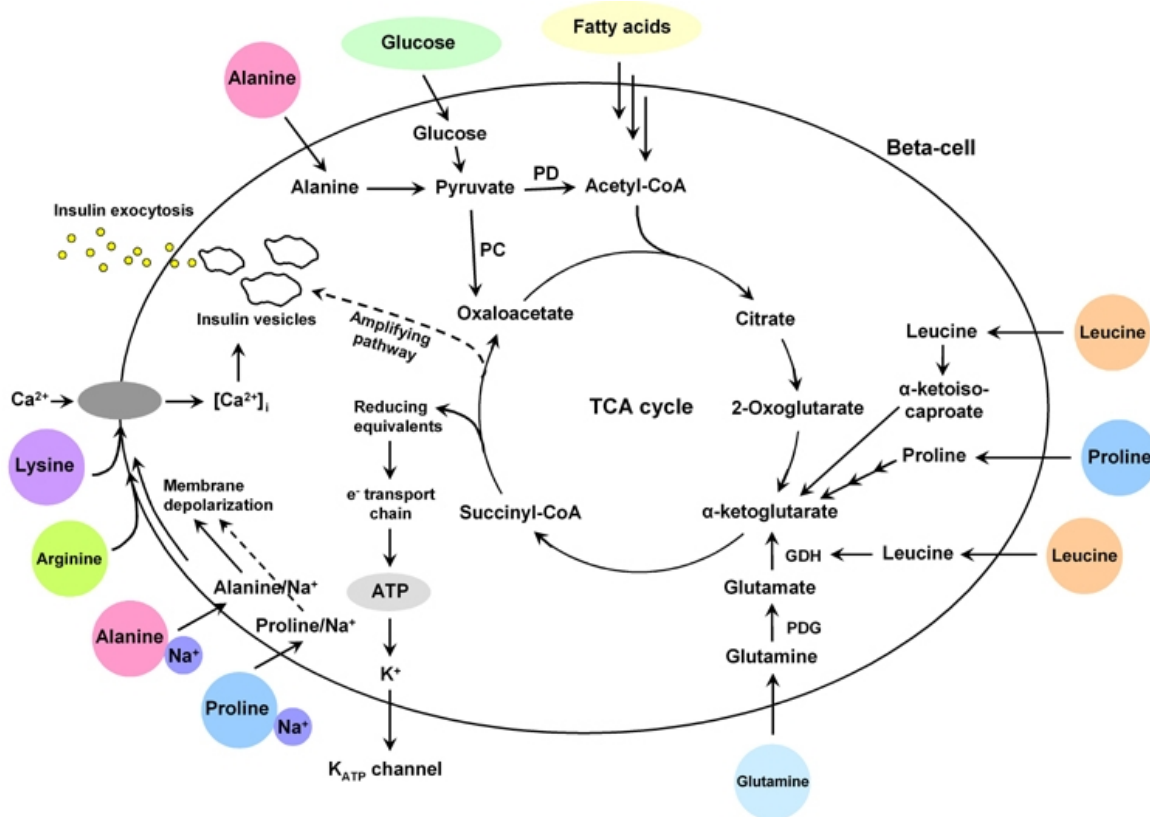


Figure 1.2.3.1: Schematic diagram of insulin secretion in β -cells stimulated by glucose, fatty acids and specific amino acids. AAs are accumulated within the β -cell by a number of different transport systems. AAs stimulate insulin secretion in β -cells through three different mechanisms: (i) metabolism with generation of ATP (L-alanine, L-proline, L-leucine, L-glutamine); (ii) co-transport with sodium resulting in membrane depolarisation (L-alanine, L-proline); (iii) uptake of cationic AAs (L-arginine, L-lysine) and membrane depolarisation. AA: amino acid. PD: pyruvate dehydrogenase. PC: pyruvate carboxylase. PDG: phosphate-dependent glutaminase. GDH: glutamate dehydrogenase. TCA: tricarboxylic acid⁴¹

1.2.3.3.1 Arginine

L – arginine is a cationic, conditionally-essential amino acid and is recognised as one of, if not the most, powerful amino acid secretagogue and is an essential synergistic compound for nutrient-induced insulin secretion¹⁴. However its effect on insulin secretion is seen only in the presence, but not absence, of glucose in isolated β -cells, indicating there is a synergistic relationship between glucose and arginine⁴². Physiologically, it is impossible for there to be a complete absence of glucose within the β -cell and therefore when single amino acids were given intravenously to healthy men, arginine produced the largest incremental increases in plasma insulin⁴⁰. Conversely, when arginine was given orally in amounts similar to that found in a large, high-protein meal, it did not stimulate a rise in plasma insulin levels when consumed either in isolation or in combination with glucose⁴³. Similar results have also been observed following low doses of arginine, equivalent to that found in commercial sports supplements⁴⁴⁻⁴⁶. On the other hand, large doses of free arginine consumed with carbohydrate as a beverage does produce large increases in both plasma arginine and plasma insulin concentration. At this dosage, however, arginine causes severe diarrhea for several hours⁴⁷.

Arginine is transported across the membrane and into the β -cell through electrogenic transport by cationic amino acid transporters (mCAT2A in mice)⁴⁸. The accumulation of this positively charged amino acid within the β -cell results in membrane depolarisation (without changing the K_{ATP} channel activity or resting membrane K^+ permeability^{42, 49}), triggering the opening of the voltage-gated Ca^{2+} channels and causing a rise in intracellular calcium, which stimulates insulin exocytosis and thus insulin secretion⁴⁸.

Furthermore, arginine may also be converted L-glutamate, generating additional metabolic coupling factors (MCF), which amplify the glucose-stimulated insulin secretion pathways and thus further enhance insulin secretion¹⁴.

1.2.3.3.2 *L-glutamine*

L-glutamine alone does not stimulate or enhance glucose-stimulated insulin secretion¹⁴.

Although glutamine is rapidly taken up by the pancreatic β -cells, it has been proposed leucine is needed to activate glutamate dehydrogenase (GDH) in order to increase glutamine entry into the tricarboxylic acid (TCA) cycle within the B-cell and thus enhance insulin secretion⁵⁰.

The production of γ -aminobutyric acid (GABA) from glutamine has been proposed as an explanation for the paradox that glutamine alone does not stimulate insulin release. When glutamine is consumed alone, it is preferentially metabolised to GABA and L-aspartate after glutamine enters the TCA cycle. Since there is no oxidation of glutamine in the process, the stimulus-secretion coupling via ATP would be minimal⁵¹.

Glutamine however, has been shown to play a prominent role in mediating insulin release in vitro when given as a mixture of amino acids in the presence of glucose. It has been hypothesised that glutamine plays a signalling role in insulin secretion, but only in the presence of elevated intracellular ATP and calcium concentrations⁵². Metabolism of glucose in the β -cell produces ATP in order to activate the rest of the glucose-stimulated insulin secretion pathway. ATP however, also inhibits glutaminolysis and drives the glutamine synthetase reaction to generate glutamine from glutamate and ammonia. The two functions work synergistically to raise the intracellular glutamine concentration. Glutamine, or its analogue DON, may increase the intracellular calcium concentration or enhance the downstream calcium signalling to amplify insulin exocytosis⁵².

Glutamine has also been shown to act as a trigger and potentiator of GLP-1 release, which is consistent with its role as the major metabolic fuel for the gut. In the murine GLP-1-secreting cell line, GLUTag, glutamine was a more potent GLP-1 (glucose-like peptide 1) secretagogue than glucose or other amino acids⁵³.

1.2.3.3.3 Intracellular L-glutamate

It has been proposed that L-glutamate promotes insulin secretion, acting as part of a metabolic coupling factor to amplify the glucose-stimulated insulin secretion pathway. Total cellular glutamate levels have been reported to increase the insulin response to glucose in human, mouse and rat islets and clonal cells⁵⁴⁻⁵⁶, although other groups have reported no changes in insulin response^{57, 58}.

Glutamate may bring about its action as an intracellular messenger, being transported into the insulin vesicles within the β -cell by glutamate transporters, where it promotes insulin exocytosis and thus insulin secretion^{59, 60}.

The glutamate-aspartate shuttle, in conjunction with the malate – α -ketoglutarate shuttle, plays a key role in the maintenance of glucose metabolism in the β -cell. The malate – α -ketoglutarate shuttle moves malate into the mitochondria in exchange for α -ketoglutarate (**FIG 2.3.2**). In the mitochondrial matrix, malate dehydrogenase (MDH) generates oxaloacetate (OOA) from malate, converting NAD to NADH in the process. OAA is then transaminated to aspartate, using glutamate as the amino group donor. Aspartate is exported into the cytosol through the inner mitochondrial membrane in exchange for glutamine, where it is converted back to OOA and then to malate, regenerating the cycle to be used by the malate- α -ketoglutarate shuttle. The synergistic relationship between the Glutamate-Aspartate shuttle and the Malate- α -ketoglutarate shuttle plays a key role in maintenance of glucose metabolism, since the shuttles allow the regeneration of NADH within the matrix, which is then oxidised into NAD and the derived electrons are transferred to the electron transport chain, creating the proton electrochemical gradient driving ATP synthesis, increasing the ADP/ATP ratio and resulting in insulin release^{15, 56}.

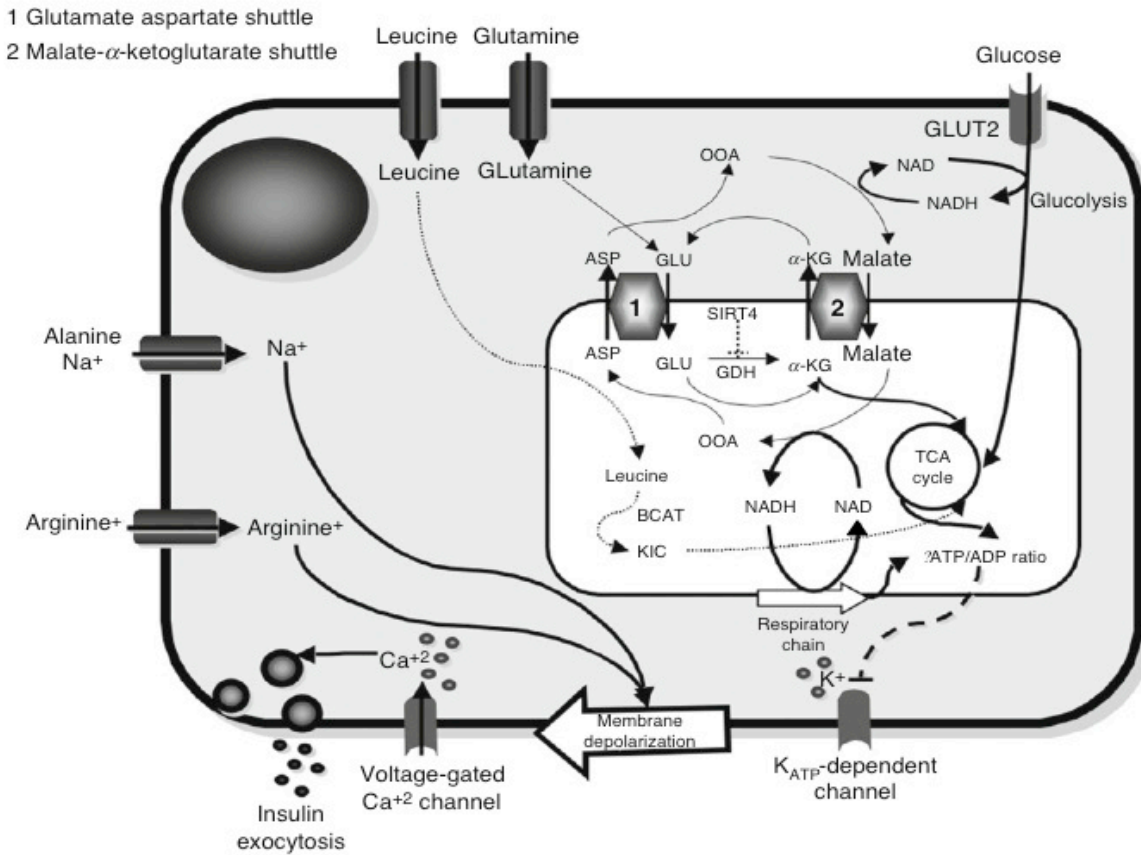


Figure 1.2.3.2: Role of the Glutamate-Aspartate and Malate- α -Ketoglutarate shuttles in amino acid-stimulated insulin secretion. The synergistic relationship between the Glutamate-Aspartate shuttle and the Malate- α -ketoglutarate shuttle plays a key role in maintenance of glucose metabolism, since the shuttles allow the regeneration of NADH within the matrix, which is then oxidised into NAD and the derived electrons are transferred to the electron transport chain, creating the proton electrochemical gradient driving ATP synthesis, increasing the ADP/ATP ratio and resulting in insulin release. ASP: Aspartate; GLU: Glutamate; α -KG: α -Ketoglutarate; OOA: Oxaloacetate.

1.2.3.3.4 L-alanine

Alanine has been shown to increase insulin secretion in isolated clonal cells⁶¹. The insulinotropic effect of alanine is reportedly a result of co-transport with sodium, which causes the depolarisation of the β -cell membrane, an increase in intracellular calcium and results in insulin secretion^{62, 63}.

The metabolism and oxidation of alanine have been shown to be important factors in its efficacy as a secretagogue⁵⁵. However prolonged exposure to alanine has previously been shown to reduce subsequent alanine-induced insulin secretion, indicating a non-linear dose-response relationship⁶¹.

1.2.3.3.5 Homocysteine

Unlike the other amino acids, homocysteine significantly inhibits both glucose- and amino-acid-stimulated insulin secretion^{14, 15, 41, 64}. The precise mechanism is not clear, however the inhibition of insulin secretion by homocysteine occurs rapidly, reversibly and in a dose-dependent manner.

Homocysteine is formed during the metabolism of methionine and could exert its inhibitory effect through interactions with key molecules to modulate enzyme activities or modify proteins⁶⁵ or cause oxidative stress damage⁶⁴. For example, homocysteine has been shown to significantly reduce the labelling of TCA cycle endpoints following glucose metabolism, which may affect the triggering and potentiation of insulin secretion.

Homocysteine also inhibits the insulinotropic effect of alanine and arginine. It has been suggested that homocysteine may also cause further damage by decreasing the bioavailability

of nitric oxide, which is needed in a constant low supply for insulin secretion and β -cell function.

1.2.3.3.6 Branched Chain Amino Acids

Branched chain amino acids (BCAA) refer to those amino acids with an aliphatic (or non-aromatic) side chain with a branch, ie a carbon atom bound to more than two other carbon atoms. This group includes leucine, isoleucine and valine.

BCAA are commonly reported to be potent secretagogues in the literature^{40, 66}, however the efficacy of the individual amino acids within the group varies despite their similar structure. For example, leucine has been shown to be among the top few amino acids producing significant insulin responses, whereas valine had minimal effect on insulin in dogs⁶⁷. Floyd et al. found similar results in humans, with leucine being among the most potent amino acids when tested on an equimolar basis⁴⁰. In both these studies, the amino acid load was administered intravenously, however the results remain consistent when leucine was ingested orally in combination with carbohydrate, with plasma leucine concentrations significantly correlating with the 2 h postprandial observed insulin response⁴⁷.

In vitro studies have also found that the addition of leucine stimulates insulin release in pancreatic β -cells⁵⁰. They revealed that leucine activates glutamate dehydrogenase activity in the β -cell, acting as both a metabolic substrate and activator. Glutamate dehydrogenase is a key enzyme controlling amino acid metabolism in the β -cells of the pancreas and its activation leads to increased glutaminolysis and a subsequent increase in the TCA cycle activity, inhibition of the ATP-regulated potassium (K_{ATP}) channel activity and thus enhanced insulin secretion^{32, 50}. In addition, leucine, or its transaminated product α -ketoisocaproate

may impact insulin secretion through direct inhibition of the K_{ATP} channel currents⁶⁸.

Dairy products, particularly whey protein, are well-known insulin secretagogues despite their comparatively low glycaemic response. The efficacy has been attributed to their high BCAA content^{33, 69}.

One of the commonly cited reasons for the efficacy of BCAAs is that their structure means they are rapidly digestible and thus the bioactive peptides and amino acids are readily available in the bloodstream^{32, 33, 69}. Just as Van Loon et al. saw with leucine, there is also a positive correlation between the postprandial insulin levels and the increase in the plasma amino acids level, with the response intensified for all BCAA, including leucine, isoleucine and valine³². This importance of digestion time is clearly demonstrated by casein and whey, two milk protein fractions. Although both proteins have high BCAA contents and high digestibility, casein coagulates when it reaches the stomach, slowing its digestion and the release of amino acids into circulation resulting in a weaker insulin response. Whey on the other hand, does not coagulate and is therefore digested faster and elicits a stronger insulin response^{33, 70}.

Another possible mechanism by which leucine may stimulate insulin secretion is through the activation of a serine and threonine kinase protein, mammalian target of rapamycin (mTOR). Activation of this protein significantly increases gene transcription and protein synthesis in pancreatic β cells, which in turn increases insulin secretion⁷¹.

1.2.4 Fat-stimulated insulin secretion

1.2.4.1 Overview

Changes in the physiological plasma free fatty acid (FFA) levels are important for regulating β -cell function and insulin release. The acute stimulatory effect of FAs on glucose stimulated insulin secretions has been demonstrated both in vitro⁷²⁻⁷⁴ and in vivo⁷⁵⁻⁷⁷.

Although fatty acids do not initiate insulin release, their presence is essential for glucose-stimulated insulin secretion¹⁴ and, at higher concentrations of FFA, they amplify the glucose-stimulated insulin response, significantly enhancing insulin response⁷⁸. This has been clearly demonstrated by Collier and colleagues^{77, 79, 80}. When fat is ingested with carbohydrate, it results in lower blood glucose levels and significantly higher insulin levels compared with those following carbohydrate ingestion alone. In one study, the addition of 37.5 g of fat to 75 g of carbohydrate resulted in a ~60% greater insulin response, compared with the carbohydrate meal alone⁸⁰. They proposed that this effect was due to the fat potentiating insulin release, possibly due to increased pancreatic insulin release combined with increased incretin hormone release (incretin hormone-stimulated insulin release discussed further in following section). At the same time as insulin levels increase, fat also slows gastric emptying and therefore the release of carbohydrate in the intestine, accounting for the lower blood glucose levels.

While acute exposure to plasma FFA substantially increases insulin secretion¹⁴ from the β -cell, low plasma FFA levels severely impair glucose-stimulated insulin secretion.¹³ This is important for normal metabolism, as insulin is required for the uptake of FFA and the synthesis of new fats. However during fasting, when plasma glucose and FFA levels are low, this excess insulin output is not required and therefore insulin release is effectively reduced.

While acute exposure (1-3 h) of pancreatic β -cells to FFA enhances insulin secretion, long-term exposure (6-24 h) to high FFA concentrations desensitises the β -cell, impairs glucose-stimulated insulin release and thus suppresses insulin secretion^{13, 14, 24}. The accumulation of triglycerides in the β -cell promotes lipotoxicity, causing β -cell dysfunction and apoptosis, reducing the β -cell mass⁸¹. Furthermore, prolonged in vitro exposure to high levels of both glucose and FFA also impairs insulin gene expression⁸².

1.2.4.1.1 Type 2 diabetes

Diets high in fat, particularly rich in saturated fatty acids, are associated with impaired insulin sensitivity and secretion, insulin resistance, hyperinsulinaemia and the development of type 2 diabetes⁸³⁻⁸⁶. Exposure to unsaturated fats also impairs glucose-stimulated insulin secretion but without causing insulin resistance⁸³.

1.2.4.1.2 Type 1 diabetes

There has been recent interest in the implications of fatty acid-modulated insulin secretion on bolus insulin requirements in type 1 diabetes. High fat meals often result in delayed hyperglycaemia in patients with type 1 diabetes as shown when insulin was dosed according to the carbohydrate content of the meal^{9, 87-89}. Lee and colleagues⁸⁹ gave 8 adults with type 1 diabetes (mean age 48 yrs) using insulin pump therapy two test meals under three conditions as follows; 1) Control meal using standard wave (24% fat); 2) High fat meal using standard wave (54% fat) and 3) High fat meal using dual wave (70% of the insulin dose given immediately, and the remaining 30% administered over a 5 h period). Postprandial glycaemia was significantly increased with the high fat meal using a standard wave bolus compared with

when the dual wave bolus was applied to the same meal. Interestingly, the postprandial glycaemic response was relatively flat for the high fat meal with the dual wave bolus, however an additional 4.7 units of insulin was administered under this condition which shows that, not only does fat change the glycaemic profile, but it also creates an additional insulin demand to maintain postprandial normoglycaemia.

In these studies, the resulting delayed hyperglycaemia is attributed to delayed gastric emptying and therefore they suggest insulin delivery patterns need to be altered to better match the glycaemic pattern (eg the use of dual or square wave patterns over a normal/standard wave using insulin pump technology)⁸⁸.

More recently, Wolpert et al. demonstrated that adults with type 1 diabetes needed additional insulin for a high fat meal compared with a low fat meal⁹⁰. A closed-loop system was used to detect the circulating blood glucose level and automatically adjust the insulin delivery in seven patients over separate 18 h periods. On average, patients required 42% more insulin following the high fat meal (60 g fat) compared to the low fat meal (10 g fat). Despite this increased insulin infusion, the high fat meal also caused more postprandial hyperglycaemia. The researchers postulated that the observed increased insulin requirement and hyperglycaemia may be due to insulin resistance caused by elevated FFA levels but acknowledged that gastric emptying and incretin hormones may also play a role.

These results were supported by Smart and colleagues, who demonstrated that increasing the fat content of the meal independently increased the postprandial blood glucose response in 33 children with type 1 diabetes³⁷. In this study the fat content of the meal was raised from 4 g to 35 g while the other macronutrients remained constant and the insulin dose was determined by the carbohydrate content of the meal (ie the same insulin dose was given under both conditions as the carbohydrate content did not vary). The mean glucose excursions after the

high fat meal were 3.4 mmol/L higher than the glucose excursions after the low fat meal between 210 to 200 minutes postprandially (0.6 mmol/L vs -2.9 mmol/L; $p = 0.01$), demonstrating there is a need for additional insulin when meals contain more fat.

In contrast to these studies, Dunn and Carroll found that fat did not alter short-term basal or postprandial insulin requirements in type 1 diabetes⁹¹. In this study, blood glucose levels and insulin requirements were determined by an artificial β -cell following 1 day (5 consecutive meals) on a high fat diet (40% fat) and 1 day on a fat-free diet (0% fat) in random order. They found that elimination of the calories from fat had no significant impact on the blood glucose response or the postprandial, basal or total insulin requirements. As the calories from fat were not replaced by another macronutrient, the two test meals were not isocaloric, which may have confounded the results.

Kordonouri and colleagues found that postprandial glycaemia was significantly lower in children and young people (6-21 yrs) with type 1 diabetes when the mealtime insulin dose was increased to account for both the fat and protein contents of the meal and a dual wave insulin delivery pattern employed to account for the delayed hyperglycaemia observed⁹². This lower postprandial glycaemic rise however, was associated with a higher risk of hypoglycaemia.

1.2.4.2 Mechanism

Although the effect of fat-stimulated insulin release is widely reported in the literature, the complete mechanism by which fatty acids stimulate insulin secretion remains unknown¹³. It is thought that fatty acids regulate insulin secretion through 3 main pathways, known as the 'trident model' of β -cell regulation¹⁴.

1) Malonyl-CoA/Long-chain CoA Signalling

FFA enter the pancreatic β -cell and are converted into long-chain acyl CoA (LC-CoA) by acyl-CoA synthase. FFA metabolism is mainly controlled by substrate supply. During fasting, LC-CoA is transported into the mitochondria, where β -oxidation of the fatty acids occurs, feeding into the TCA cycle and leading to insulin exocytosis. LC-CoA is also thought to be an effector molecule in signal transduction within the β -cell as it can also a) activate the Protein Kinase C (PKC) signal transduction pathway involved in GSIS, b) interact with the K_{ATP} channel to close it, c) stimulate calcium influx through the voltage-gated calcium channels or calcium mobilisation from intracellular stores and d) directly activate and enhance insulin exocytosis⁷⁸. At fasting blood glucose levels however, this fatty acid metabolism has little impact on insulin levels and primarily assists in maintaining basal insulin secretion^{13, 14}. Conversely, when high levels of glucose are available, glycolysis occurs, creating Acetyl-CoA, which feeds into the TCA cycle and upregulating the glucose-stimulated insulin secretion. However, Acetyl-CoA also subsequently produces Malonyl-CoA, which inhibits the β -oxidation of LC-CoA. Therefore Malonyl-CoA effectively switches off β -cell fatty acid metabolism, leading to an increase in cytosolic levels of LC-CoA, which potentiates glucose-stimulated insulin secretion through a variety of mechanisms, ultimately activating insulin exocytosis. Therefore, in the presence of both glucose and fatty acids insulin release is enhanced^{14, 78}.

2) Fatty acids and lipid receptor signaling

FFAs are ligands for a small group of G-protein-coupled transmembrane receptors, including GPR40, which is selectively expressed in β -cells. Long-chain fatty acids, including palmitate, oleate, stearate, linoleate and linolenate, bind to and activate GPR40, resulting in increased

intracellular calcium levels. This occurs through calcium influx via the voltage-gated calcium channels and mobilisation of intracellular calcium stores and consequently stimulates insulin exocytosis⁷⁸.

3) Triglyceride/FFA cycling

In the presence of high glucose levels, both lipolysis of intracellular triglycerides and fatty acid esterification are promoted within the β -cell⁹³. Elevated glucose increases lipolysis of intracellular triglycerides, resulting in increased levels of LC-CoA, diacyl-glycerol, phospholipids and FFAs. With β -oxidation blocked by malonyl-CoA, these intermediates build up in the cytosol and can act as lipid signalling molecules to amplify glucose-stimulated insulin secretion. Furthermore, LC-CoA can be esterified with glycerol-3-phosphate (Glyc-3-P) to form new triglycerides, diacyl-glycerols and phospholipids and thus complete the cycle (**FIG 2.3.1**). There are two possible advantages of this triglyceride/FFA cycling pathway. Firstly, this pathway generates lipid-signalling molecules, which amplify the glucose-stimulated insulin secretion. Secondly, high flux of glucose through mitochondrial oxidation generates superoxides and free radicals, which damage the β -cell. Therefore triglyceride/FFA cycling presents an alternative pathway for glucose that bypasses the need for mitochondrial oxidation and can therefore protect the β -cell and preserve the pancreatic β -cell mass⁹³.

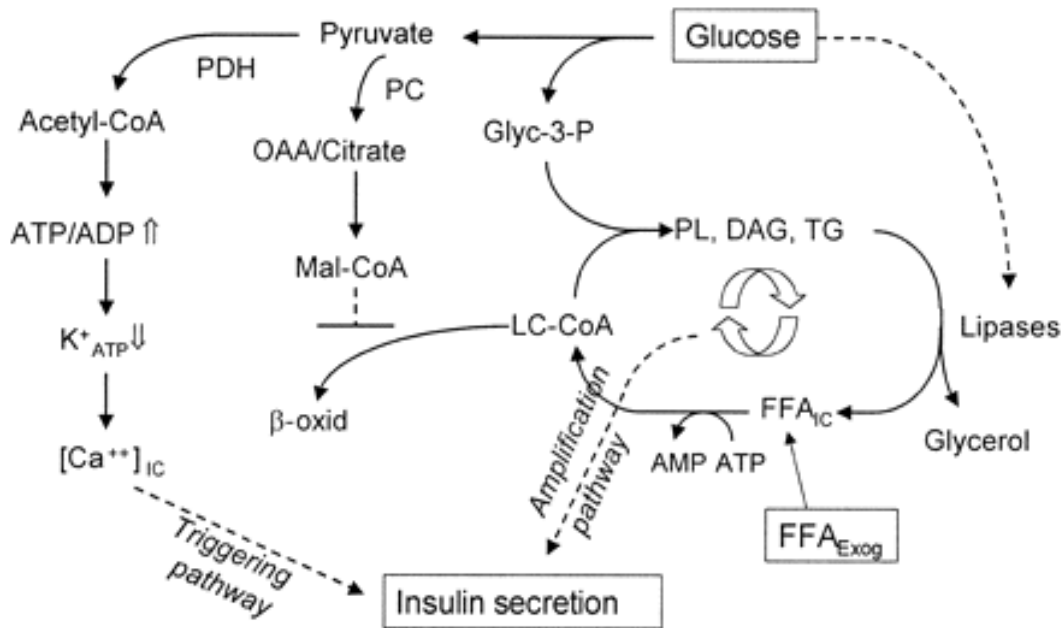


Figure 1.2.4.1: Fatty acid-stimulated insulin secretion⁹³

Chronic exposure of the β -cell to elevated FFA desensitises the β -cell and suppresses insulin secretion. High levels of saturated fatty acids over long periods of time impairs glucose oxidation, resulting in a fall in the ATP/AMP ratio, reduction in FFA synthesis and promotion of FFA oxidation which impairs glucose-stimulated insulin secretion. Furthermore, this increased FFA oxidation also inhibits the generation of the lipid-derived signalling molecules, further reducing insulin secretion (see triglyceride/FFA cycling pathway)^{13, 14}. A number of possible mechanisms have been proposed for the effect of chronic exposure to FFA on glucose-stimulated insulin secretion including the induction of UCPs (uncoupling proteins), GLUT2, glucokinase and insulin gene expression⁹⁴. In rat models, β -cell dysfunction is related to triglyceride accumulation within the islets, which increased the production of nitric oxide, ceramide synthesis and promoted β -cell apoptosis⁸¹.

1.2.4.3 Effect of different fat sources

The potency of fatty acids on insulin secretion are directly correlated with the fatty acid chain length and degree of unsaturation^{72, 76}.

1.2.4.3.1 Chain length

Stein and colleagues found there was positive correlation between fatty acid chain length and insulin response, with long chain fatty acids, such as palmitate (16:0) and linoleate (18:2), acutely increasing glucose-stimulated insulin secretion, whereas short- and medium-chain fatty acids and saturated fatty acids inhibited insulin secretion⁷⁶. Hosokawa et al. also observed that long chain fatty acids such as palmitate (16:0) and linoleate (18:2) and linolenate (18:3) potentiate insulin secretion in response to basal glucose concentrations in vitro⁹⁵. In contrast however, Opara and colleagues reported that as the fatty acid chain length increases there is a bell-shaped insulin response curve, with peak insulin release at 12 carbon atoms (Lauric acid; 12:0), no insulin response with 6 and 16 carbon atoms (hexanoic acid; 6:0 and Palmitic acid; 16:0) and an inhibitory effect on insulin at both 4 and 18 carbon atom chain lengths (Butanoic acid; 4:0 and Stearic acid; 18:0) (**FIG 2.3.2**)⁷². These conflicting results with regards to insulin secretion and LCFA, may be due in part to a lack of carefully conducted dose-response studies and/or disparities in the cell species and/or fatty acid concentrations used.

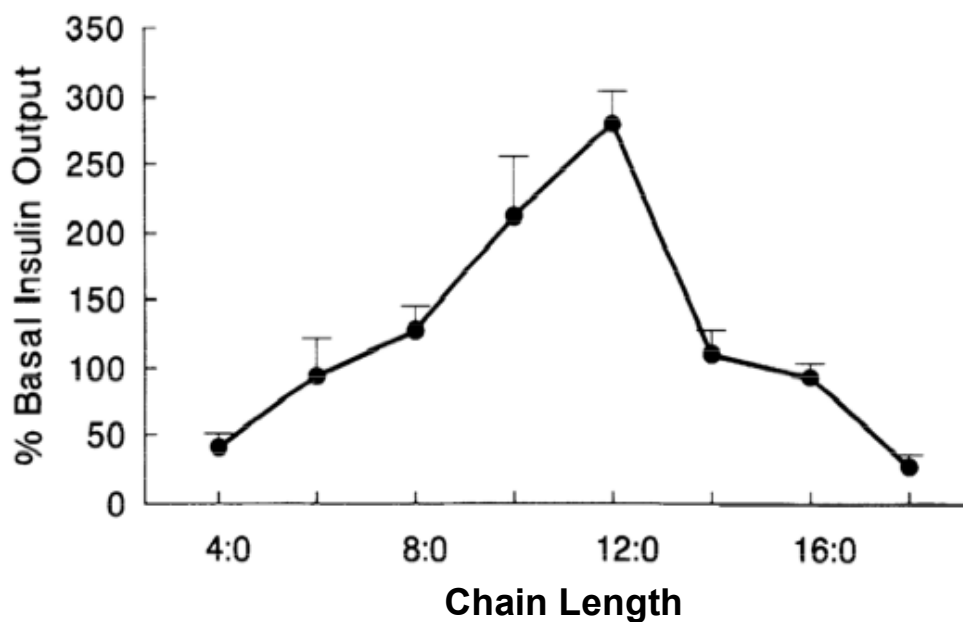


Figure 1.2.4.2: Insulin release is augmented by the fatty acid chain length⁷²

1.2.4.3.2 Saturation

The degree of saturation may affect the insulinotropic properties of fatty acids, although the results are controversial. Studies in vivo have found that the postprandial insulin response was essentially unaffected by the type of fat (unsaturated vs. saturated) consumed in healthy subjects^{96,97}. Alternatively, some studies have found that acute exposure of the pancreatic β -cell to both high glucose concentrations and saturated free fatty acids results in substantial increase in insulin release^{13,73,74,98}. Furthermore, while the insulin response was significantly increased by adding 50 and 100 g of butter, replacing it with 40 and 80 g of olive oil (monounsaturated fat) had no effect⁹⁹.

In contrast, Lardonis and colleagues found that polyunsaturated fatty acids significantly increased serum insulin responses by 62% compared to saturated fat. This finding is

supported by research by Stein et al. who reported that polyunsaturated fatty acids (such as linoleic, linolenic and arachidonic acids) stimulate insulin secretion in vitro while saturated fatty acids (such as palmitic acid) inhibited insulin secretion in pancreatic cells isolated from 4 h starved rats⁷⁶. Another study suggested monounsaturated fatty acids may be more effective secretagogues, with the insulin response to a meal high in monounsaturated fatty acids but low in polyunsaturated omega-6 fatty acids significantly higher compared to that following a meal with a low ratio of monounsaturated to polyunsaturated fatty acids¹⁰⁰.

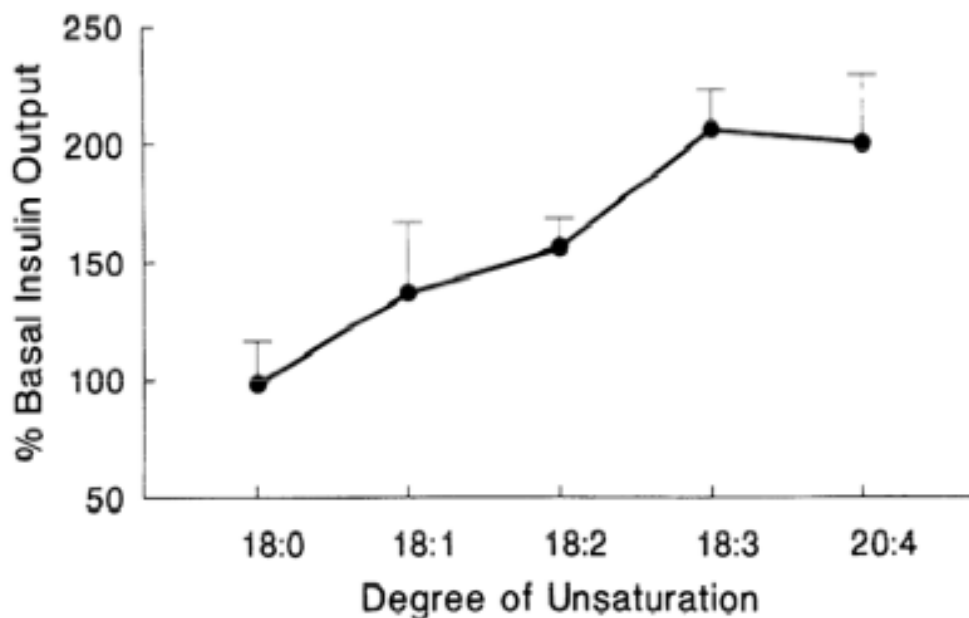


Figure 1.2.4.3: Insulin release is augmented by the degree of fatty acid unsaturation⁷²

1.2.5 Incretin hormone-stimulated insulin secretion

1.2.5.1 Introduction

Insulinotropic peptides are secreted into the intestines in response to nutrients and enhance insulin secretion. Glucagon-like peptide-1 (GLP-1) and Glucose-dependent insulinotropic

polypeptide (GIP) are the two incretin peptides and are rapidly secreted into the hepatic portal venous system by specific enteroendocrine cells located in the intestinal mucosa in response to nutrient ingestion.¹⁰¹

Insulin secretion stimulated by these incretins is often referred to as the 'incretin effect' and accounts for around 50% of the total insulin secreted in response to oral glucose¹⁰². The magnitude of their effect on insulin secretion is dose-dependent and is proportional to the calorie content of the meal and the type of macronutrient ingested. They are most strongly activated by glucose and to a lesser extent by fatty acids while amino acids are a relatively weak stimulus¹⁰¹.

Both GIP and GLP-1 exert their insulinotropic effect only in the presence of elevated blood glucose levels and thus are glucose dependent¹⁰². This prevents excess insulin secretion during fasting and following meals without carbohydrate, thus avoiding hypoglycaemia. When glucose is given orally it induces a greater insulin response than when glucose is given intravenously, even if the blood glucose response was higher with the intravenous glucose¹⁰³⁻¹⁰⁵.

The early increase in glucose-stimulated insulin secretion following fat ingestion is postulated to be due to the rise in incretin hormones⁸⁰. Fatty acids stimulate the release of GIP and GLP-1, however without glucose, the incretin secretion is not sufficient to stimulate insulin secretion from the β -cells at fasting blood glucose levels. Unsaturated fats, such as olive oil have been shown to induce higher concentrations of GLP-1 and GIP than saturated fats such as butter.⁹⁷

Although a relatively weak stimulus in comparison with glucose and fat, protein in the intestinal lumen also stimulates the release of incretin hormones, potentiating insulin

secretion. The resulting insulin response is largely due to the presence of protein, or digestion products of protein, in the intestine rather than the total amino acid concentration in the circulation.¹⁰⁶

1.2.5.1.1 Type 2 diabetes

Incretin hormones have recently become a target for type 2 diabetes therapies. GIP and GLP-1 release are severely impaired or absent in type 2 diabetes, which is likely to contribute to worsening insulin secretion and glycaemic control¹⁰⁷. Treatment with continuous, subcutaneous infusions of GLP-1 for 6 weeks has been shown to substantially improve the first-phase insulin response, decrease plasma glucose, HbA1c, fructosamine and free fatty acids and improve insulin sensitivity and β -cell function in patients with type 2 diabetes^{108, 109}.

1.2.5.2 Mechanism

GIP and GLP-1 are synthesised in the enteroendocrine K- and L-cells respectively of the intestines. The K-cells are primarily located in the proximal small intestine (duodenum and jejunum), whereas the L-cells are mainly located in the distal ileum and colon. They are open-type intestinal epithelial endocrine cells that are in direct contact with nutrients through the microvilli on the luminal surface¹¹⁰. The incretin hormones are stored in granules within the cell until the presence of nutrients in the intestines stimulates their release.

Fasting plasma levels of GIP and GLP-1 are low but increase rapidly within just a few minutes after a meal¹⁰². Their release follows a biphasic pattern, with an early phase beginning within 5-15 min and a prolonged second phase following within 30-60 min¹¹¹. This early phase release occurs within 5-15 minutes may be due to the “cephalic” or

“preabsorptive” phase insulin release, whereby GLP-1 and GIP release is stimulated by neural factors¹¹². This mechanism has not been established in humans, however it is thought to be mediated, at least in part, by cholinergic mechanisms. The latter phase of this biphasic release pattern may be due to the movement of food through the digestive system and the nutrients that are released at various stages coming into contact with the K- and L-cells along the intestines¹⁰².

The incretin hormones achieve their insulinotropic effects by binding to their specific receptors, GIPR and GLP-1R, on the β -cell. This activates adenylyl cyclase, which produces cyclic adenosine monophosphate (cAMP) and subsequently activates Protein Kinase A (PKA). Activation of this signalling pathway within the β -cell has a number of roles including closing the K_{ATP} channels, elevating the intracellular calcium concentration, increasing mitochondrial ATP synthesis, all of which ultimately enhance exocytosis of insulin from insulin-secretory vesicles¹¹³.

GLP-1 also influences insulin secretion indirectly by inhibiting gastric emptying¹¹⁴, decreasing appetite and food intake^{114, 115}, inhibiting glucagon secretion^{116, 117} and slowing the rate of endogenous insulin production¹¹⁸ all of which lower blood glucose levels.

GIP and GLP-1 also promote β -cell mass and function by reversing the age-dependent decline in β -cell function, protecting the β -cells from apoptosis and stimulating cell proliferation and neogenesis¹¹⁹⁻¹²¹.

Both GIP and GLP-1 work to acutely increase insulin secretion following meal ingestion but are then degraded by DPP-4. Their half-life is between 1.5 to 2 min¹⁰².

1.3 Insulin dosing in type 1 diabetes

Type 1 diabetes is an autoimmune disorder resulting in an absolute endogenous insulin deficiency and is characterised by chronic hyperglycaemia (ie elevated plasma blood glucose levels). Current medical management revolves around exogenous insulin therapy to restore blood glucose levels to within an optimal range. At present there is no cure for type 1 diabetes, therefore effective strategies to assist in the achievement and maintenance of normoglycaemia are required to promote the acute and long-term health and wellbeing of individuals with type 1 diabetes.

1.3.1 Exogenous insulin therapy

Exogenous insulin is designed to be injected or infused subcutaneously as hexamers of insulin crystalized around a zinc molecule. These hexamers need to dissociate from one another and diffuse from the injection site into the blood stream, thereby limiting their rate of absorption, and thus the time to onset of action. Exogenous insulins can be classified into several categories based on their time to onset and peak and their duration of action. Rapid acting insulin takes up to 15 minutes to begin exerting an effect on glycaemia and 30-90 minutes to reach its peak action. Unlike the relatively short half-life of endogenous insulin (5-7 minutes), exogenous insulin will still be acting in the system for 3-5 hours, so bolus insulin dosing decisions will have an impact on blood glucose levels for several hours. Alternatively, long-acting or 'basal' insulin begins acting after 1-2 h and is designed to be 'peakless', gradually releasing insulin for up to 24 h.

There are two modes of insulin delivery, multiple daily injections (MDI) using a syringe or insulin 'pen', a cartridge of insulin stored in a pen-shaped device with a needle for injections,

or continuous subcutaneous insulin infusion (CSII), where insulin is delivered continuously from a cartridge in a programmable, pager-sized insulin pump through a fine cannula placed in the subcutaneous tissue. The type of insulin required is determined, in part, by the mode of insulin delivery. MDI usually requires a 'basal-bolus' regimen, where a long-acting insulin is injected 1-2 times per day (depending on the brand) to simulate normal basal insulin release and then a rapid-acting insulin is used to cover meals and correct high blood glucose levels. Since CSII delivers a continuous stream of insulin, only a rapid-acting insulin is required. The insulin pump can be programmed to deliver small amounts of insulin continuously and then larger 'boluses' for meals and to correct blood glucose levels. Insulin pumps can deliver very accurate and precise doses, down to one tenth of a unit of insulin, whereas insulin pens can only deliver whole or half units. Insulin pumps are also advantageous in that they can be programmed to adjust the basal rate of insulin over the day for normal changes in insulin sensitivity and can calculate the insulin dose required based on the pre-programmed insulin: carbohydrate ratio (ICR; discussed in greater detail in the follow section) and the correction factor (CF) for correcting high blood glucose levels to a set target. Furthermore, insulin pumps allow insulin to be delivered in 3 types of patterns, a 'normal' or 'standard' bolus, where the total bolus is delivered immediately, a 'dual wave' bolus where a proportion of the insulin is delivered immediately and the remainder delivered over a selected period of time and a 'square wave' or 'extended' bolus, where the total insulin dose is delivered evenly over a set period of time. These delivery patterns can be matched to different meal types, however no clear, evidence-based guidelines currently exist for when and how to use these features. Since the insulin is delivered continuously an insulin pump, adjustments can be made throughout the day for factors that affect blood glucose levels including physical activity and alcohol intake. In contrast, people using MDI need to take their basal insulin 1 or 2 times per

day and therefore do not have the flexibility to adjust their rates over the day for change in insulin sensitivity or lifestyle.

Advancements in insulin therapy to more closely mimic normal physiological insulin secretion, in terms of both the types of insulins available and the modes of delivery, offer improvements in glycaemic control. The challenge lies in how the lessons learnt from normal, postprandial insulin secretion can be applied to optimise mealtime insulin therapy in type 1 diabetes.

1.3.2 Glycaemic control in type 1 diabetes

Achieving and maintaining optimal glycaemic control is considered a key goal of diabetes management in order to reduce the risk of diabetes-related complications¹²².

As red blood cells turn over approximately every 120 days, the level of glycated haemoglobin A1c (HbA1c) provides a measure of mean plasma blood glucose over the previous 3 months and is the gold standard measure of longer-term glycaemic control. For people with type 1 and type 2 diabetes, the American Diabetes Association recommends an HbA1c level of less than 7% for non-pregnant adults, but highlights this target needs to be individualised¹²². This recommendation is based on the Diabetes Control and Complications Trial (DCCT), which showed an HbA1c at or below 7% was associated with a reduced risk of microvascular diabetes complications¹²³. An HbA1c of 5% translates to a mean blood glucose level of 5.4 mmol/L, while an HbA1c of 7% translates to a mean blood glucose level of 8.6 mmol/L.

Postprandial blood glucose levels are key targets for acute and chronic glycaemic control. Postprandial hyperglycaemia spikes have been shown to be a direct and independent risk factor for CVD¹²⁴. The American Diabetes Association recommend that peak postprandial

blood glucose levels should not exceed 10 mmol/L¹²². Strategies for adjusting insulin doses for meals will be discussed in detail later in this section.

Recently there has been a great deal of interest in the importance of glycaemic variability for reducing the risk of diabetes-related complications, and in particular, cardiovascular disease. Studies have shown that oxidative stress and epithelial dysfunction are closely related to both chronic hyperglycaemia and the degree of fluctuation in blood glucose¹²⁵. Nonetheless, analysis of the DCCT data suggested that glycaemic variability had no impact on the risk of microvascular complications¹²⁶, although this analysis considered only the area under the blood glucose curve and the standard deviation around the mean blood glucose level. There are other measures of glycaemic variability, which may be more informative.

Continuous glucose monitoring systems (CGMS), which sample interstitial blood glucose levels every minute and record the mean every 5 minutes, provide detailed insights into fluctuations in glycaemia. Several measures of blood glucose variability have been suggested in the literature but there is great debate over their statistical and clinical appropriateness¹²⁷⁻¹³⁰. The mean amplitude of glycaemic excursions (MAGE) has been suggested as the most appropriate and comprehensive tool as it denotes the summed mean differences between consecutive peaks and nadirs in blood glucose levels for differences greater than 1 standard deviation of the mean. In doing so, the MAGE is not dependent on the mean blood glucose level nor defined targeted blood glucose levels and only considers large fluctuations in glycaemia, whilst excluding minor ones^{127, 128}. MAGE has been directly linked with the risk of microvascular complications, with univariate analysis showing MAGE to be a strong and significant predictor of oxidative stress ($r = 0.8863$, $p < 0.05$)¹³¹.

1.3.3 Historical perspective to mealtime insulin dosing

Historically, insulin therapy has been inflexible, with nutrition education primarily focused on maintaining a prescribed food intake matched to a rigid insulin regimen. A number of different theoretical approaches to quantify and categorise foods have been used in practice, including food exchanges, calorie/carbohydrate points and total available glucose^{5, 132}.

The ‘total available glucose’ approach was developed soon after the discovery of insulin in 1921. Foods were counted based on the total amount of glucose supplied to the body, with 100% of carbohydrates, 58% of protein and 10% of fat converted to available glucose. The overarching goal was to maintain a constant intake of available glucose with a matched insulin regimen¹³³⁻¹³⁵.

The Diabetes Control and Complications Trial (DCCT) was a landmark study examining the determinants and effects of intensive insulin therapy. Four dietary approaches for insulin adjustment from around the world were used during the study, comprised of healthy food choices, exchanges for meal planning, carbohydrate counting and total available glucose⁵.

The DCCT showed that individuals who reported *always* adjusting their insulin based on what they ate, had a HbA1c 0.5% lower than those who *never* adjusted their insulin¹³⁶. However, the study does not differentiate among the effectiveness of the different dietary approaches.

The ‘food exchanges for meal planning’ approach was introduced in the United States in 1950 and was commonly used in the United States during the DCCT. Its popularity fell following the DCCT as carbohydrate counting became increasingly popular but it is still in use. Foods are categorised into starch, vegetable, milk, fruit, meat, and fat exchanges and subdivided based on their carbohydrate, protein, fat and calorie contents. For example a food classed as a low fat milk exchange contains 12 g carbohydrate, 8 g of protein, 5 g of fat and 120 calories. This

allows foods in a meal plan to be exchanged for similar foods, while maintaining a consistent intake. Insulin dose is calculated based on the number of starch, milk and fruit exchanges.

The American Diabetes Association (ADA) published the first food exchange tables in the 1950's¹³⁷. The exchanges for meal planning approach has long been recognised as a complex concept requiring a higher educational status¹³² as the system requires a detailed knowledge of food composition to determine the correct number and type of exchanges eg a glazed doughnut is classed as 2 carbohydrate and 2 fat exchanges, while a plain doughnut is 2 carbohydrate but only 1 fat exchange, and to exchange foods in a meal plan while meeting an overall daily goals. The ADA has since revised the original food exchange tables with an increasing focus on carbohydrate, and thus simplifying the approach.

The carbohydrate counting approach adopted in the Diabetes Control and Complications Trial (DCCT), classes carbohydrate foods into portions equating to 10 or 15 g of carbohydrate, referred to as a carbohydrate exchange. At the time of the DCCT, this was the primary dietary strategy for diabetes in the United Kingdom but was relatively uncommon in the United States and its validity and reliability was challenged. This carbohydrate exchange approach allowed a set number of carbohydrate exchanges to be prescribed per meal/snack, promoting a consistent carbohydrate intake and matched insulin regimen, however gave patients the freedom to select different carbohydrate foods up to the same exchange value.

With developments in medical technology and a shift towards patient-centered care, insulin therapy has become more flexible and thus medical nutrition therapy has also needed to change from rigid dietary prescriptions designed to maintain consistent food intake to nutrition education to assist patients to match their insulin to flexible food intakes and patterns.

1.3.4 Carbohydrate counting

Carbohydrate counting has long been considered the cornerstone of intensive insulin therapy, with bolus insulin doses matched to the carbohydrate content of the meal¹³⁸. This practice is based on the premise that carbohydrate is the predominant macronutrient contributing to the rise in post-prandial blood glucose levels.

Modern carbohydrate counting can be difficult to define as it has evolved and encompasses multiple tiers reflecting the increasing complexity with more advanced methods.

Carbohydrate counting ranges from an awareness of carbohydrate-containing foods and their impact on blood glucose levels through to counting the number of carbohydrate exchanges or grams of carbohydrate eaten and using an insulin:carbohydrate ratio to calculate the bolus dose required¹³⁹.

Current diabetes management guidelines around the world recommend that carbohydrate counting should be used to adjust mealtime insulin in both children and adults with type 1 diabetes. A review undertaken by the ADA in 2008, concluded that “Individuals receiving intensive insulin therapy should adjust their pre-meal insulin dosages based on the carbohydrate content of meals”¹³⁸. This review forms the evidence base for the ADA’s Standards of Medical Care, which states that “Monitoring carbohydrate, whether by carbohydrate counting, choices, or experience-based estimation, remains a key strategy in achieving glycaemic control¹⁴⁰” and that “Most people with type 1 diabetes should be educated in how to match prandial insulin dose to carbohydrate intake, pre-meal blood glucose, and anticipated activity”¹²². The Australian Paediatric Endocrine Group and Australian Diabetes Society’s ‘National Evidence-Based Clinical Care Guidelines for Type 1 Diabetes in Children, Adolescents and Adults’ found similar results, concluding that the

“matching of meal-time insulin dose to carbohydrate intake should be considered for patients using multiple daily injection therapy”¹⁴¹.

These recommendations are based simply on narrative review and grading of the limited available evidence. Although carbohydrate counting is widely endorsed as the gold standard in dietary management of type 1 for glycaemic control, the overall effectiveness of the strategy has not been assessed as a benchmark for other strategies or for health professionals in practice at this point in time.

1.3.4.1 Evidence for carbohydrate counting

1.3.4.1.1 Scientific Basis for carbohydrate counting

Thirty years ago, Slama et al. showed a significant correlation between the amount of carbohydrate consumed and the dose of insulin needed to restore blood glucose levels using an artificial pancreas²¹. Further work confirmed the linear relationship between carbohydrate intake and insulin requirement^{22, 142, 143}. However, the underlying theoretical basis and its practical utility have since been questioned^{3, 5, 7, 144, 145}. Bao et al., for example, showed that available carbohydrate could explain much of the variance in glucose response to iso-caloric portions of single foods, but was not a significant predictor of the response to mixed meals containing variable amounts of carbohydrate, fat and protein¹⁶. Moreover, the same amount of available carbohydrate from different food sources is known to produce significantly varying blood glucose responses in both healthy and diabetic subjects^{3, 10, 11}. Indeed, the predictable difference in responses to different carbohydrate-containing foods is the basis of the glycaemic index (GI). In meta-analyses, low GI diets have been shown to improve HbA1c compared with conventional or high GI diets¹⁴⁶.

Schrezenmeir et al, highlighted one of the key underlying problems with carbohydrate counting in their 1989 paper⁷. Carbohydrate counting is based on the premise that carbohydrate is the predominant macronutrient raising blood glucose levels. Since the primary function of insulin is to lower blood glucose levels, exogenous insulin doses for meals in type 1 diabetes are dosed according to the predicted rise in blood glucose levels based on the carbohydrate content of the meal. However as Schrezenmeir et al. identified, the glycaemic response to 3 carbohydrate breakfasts in healthy subjects differed significantly to the insulin requirements of subjects with type 1 diabetes, thus highlighting the inherent flaw in carbohydrate counting⁷.

The underlying problem in type 1 diabetes is the lack of endogenous insulin production. It would therefore be logical that exogenous insulin doses should be matched to the normal endogenous insulin response, rather than the predicted rise in blood glucose level. As elucidated in the previous section, a number of nutritional factors, including protein and fat, also modulate postprandial insulin secretion. Therefore dosing insulin solely on the basis of the carbohydrate content of the meal reflects a narrow scope of focus within a bigger picture.

1.3.4.2 What improvement in glycaemic control can be achieved with carbohydrate counting?

There is a paucity of studies assessing the effectiveness of carbohydrate counting for glycaemic control in type 1 diabetes. This lack of evidence suggests carbohydrate counting was integrated into clinical practice before the evidence base was well established. There is a belief amongst the diabetes community that carbohydrate counting is a well-founded, evidence-based therapy.

The Diabetes Control and Complications Trial (DCCT) is often cited as key evidence for carbohydrate counting as it is a landmark, international trial. As discussed earlier in this chapter, the DCCT used 4 dietary approaches for insulin adjustment from around the world to examine the determinants and effects of intensive insulin therapy one of which as carbohydrate counting⁵. Although the DCCT showed that those who reported always adjusting their insulin (on the basis of food eaten) had an HbA1c 0.5% points lower than those who never adjusted their insulin¹³⁶, the publications did not differentiate between the effectiveness of the different dietary approaches so the effectiveness of carbohydrate counting alone cannot be elucidated. Furthermore, this intervention was relatively uncommon in the USA at the time, and its validity and reliability were questioned by physicians⁵.

Two of the most well-established carbohydrate counting programs is the Diabetes Teaching and Treatment Program (DTTP) and the Dose Adjustment For Normal Eating (DAFNE) program. The DTTP was first developed in Germany in 1978 and are now standard practice for adults with type 1 diabetes in Germany and Austria. It is a structured 5-day training program teaching flexible insulin therapy so that mealtime insulin doses can be adjusted according to the carbohydrate content of meals and thus allowing dietary freedom. In 2005, Samann et al published the results from over 9,500 adults who participated in the program, which showed that the DTTP improved glycaemic control without increasing the risk of hypoglycaemia¹⁴⁷. While both the DTTP is well-known and is widely published with large sample sizes, the studies were designed as program evaluations rather than clinical trials and therefore lack control groups for comparison.

The DTTP was translated and adapted for the United Kingdom and renamed the DAFNE program. A 2002 trial in 3 centres, revealed an improvement in HbA1c of 1% point (11 mmol/mol) at 6 months and 0.5% point (5 mmol/mol) at 12 months. There were no changes

in weight or risk of severe hypoglycaemia but there were considerable improvements in participants' quality of life¹⁴⁸. Since this trial, the DAFNE has been rolled out across the UK and Australia.

There is conflicting evidence regarding the efficacy of carbohydrate counting. Some studies have shown a beneficial effect with carbohydrate counting in adults¹⁴⁹⁻¹⁵², however like the DTTP, not all studies^{151, 152} included a control group making interpretation difficult. Other studies have shown no significant difference between carbohydrate counting and general dietary advice¹⁴⁴ or empirical methods¹⁵³. Two studies, 1 in children¹⁵⁴ and 1 in adults¹⁵⁵ found alternative methods superior to carbohydrate counting for glycaemic control.

Gilbertson et al, compared carbohydrate counting with flexible, unmeasured low GI dietary advice in children aged 8-13 years¹⁵⁴. At 6 months there was no difference in glycaemic control between the 2 groups but at 12 months HbA1c was 0.6% lower in the low GI group ($p = 0.05$). Furthermore 64% of children and 91% of their parents who had used both methods of adjusting insulin doses, reported they preferred the low GI diet to carbohydrate counting ($p < 0.01$ and $p < 0.001$ respectively).

In 2009, Mehta et al. showed that greater accuracy and precision in parent's ability to count carbohydrates was associated with a lower HbA1c in their child; however only precision was a significant predictor of HbA1c ($p = 0.9$ and $p = 0.02$ respectively). The child's HbA1c was 0.8% points lower when their parents were above the 75th percentile for precision. However, there is great debate within the diabetes community over how precisely carbohydrates need to be counted to achieve good glycaemic control. There are several commonly used methods including counting in 1 gram increments, 10 g portions and 15 g exchanges. Each method has its own advantages for example, counting in 10 g portions makes reading food labels easier as carbohydrate values are easily divisible into 10 portions, whereas common food portions

contain approximately 15 g carbohydrate, such as 1 slice of white bread, 1 medium sized apple and ½ cup of juice etc.

At present there is a lack of evidence to support the use of one method of carbohydrate counting over another. It is a widely held belief among patients and health professionals that counting in gram increments is more accurate and leads to better blood glucose control than less precise measures such as 10 g portions or 15 g exchanges. However counting carbohydrates this precisely may be unnecessary. Smart et al. have shown that children and adolescents using intensive insulin therapy can maintain glycaemic control without precise carbohydrate counting. Their studies demonstrated that postprandial glycaemic control was maintained with a 10 g variation in carbohydrate content with an individualised insulin: carbohydrate ratio¹⁵⁶ but not with a 20 g variation¹⁵⁷. These studies concur with that of Shapira et al¹⁵⁸, who found that a similar proportion of blood glucose levels were within the normal range when carbohydrate was estimated to the nearest gram or when a 15 g carbohydrate exchange was used. Furthermore, individuals who could accurately estimate the carbohydrate content of meals and snacks within 10 g of the true value had the lowest HbA1c¹⁵⁹. Indeed, children who counted in gram increments of carbohydrate did not estimate the true carbohydrate content of tested meals and snacks with greater accuracy than those using 10 g or 15 g exchanges¹⁶⁰.

One of the key limitations of carbohydrate counting is the accuracy with which people can estimate the true carbohydrate content of their meals. The literature shows a wide variation in this ability, with studies showing most subjects were able to accurately estimate carbohydrate to within 10-15 g¹⁶⁰ or within 15-20% of the true value^{4, 161}, while other studies revealed only around half or fewer could accurately estimate carbohydrate content^{159, 162} or had large variations in estimations¹⁵⁸.

A cross-sectional, observational study in 48 adolescents with type 1 diabetes aged 12-18 years showed that only 23% of adolescents could estimate the carbohydrate content of common meals within 10g of the true value¹⁵⁹. The participants were asked to assess the amount of carbohydrate in 32 breakfasts, lunches, dinners and snacks commonly consumed by children of that age. The meals were presented either as food models or as actual food and in some cases were presented as standard serve sizes eg packaged snacks, while other meals were self-served by study participants. The proportion of children who could identify the carbohydrate within 20 g increased to 31% and to 52% when the level of precision was dropped to within 30 g of the true value. This study highlights some of the practical limitations of asking children to count carbohydrates in more precise increments.

As highlighted above, this finding is not unanimous in the literature. Another study revealed that 73% of children could accurately estimate the carbohydrate content of meals and snacks within 10-15 g of the true value¹⁶⁰. This study included slightly younger children from age 8 through to adolescents aged 18 but like the previous study, included real foods representing commonly consumed breakfasts, lunches, dinners and snacks. The authors found that meals served in non-standard quantities such as rice, pasta were frequently underestimated, while snacks such as fruit and baked foods were often overestimated.

1.3.4.3 Nutritional implications of carbohydrate counting

The potential adverse nutritional implications of carbohydrate counting should not be ignored. Carbohydrate counting has been linked with unhealthy food beliefs^{163, 164}, an increased reliance on packaged foods as the carbohydrate content is published on the nutrition information label^{163, 165} and high intakes of fats and protein exceeding nutritional recommendations in an effort to avoid insulin¹⁶⁵⁻¹⁶⁷. Studies have shown that children and

adolescents with type 1 diabetes are at greater risk of atherosclerosis¹⁶⁶, dyslipidaemia and CVD¹⁶⁵, possibly due to avoidance of carbohydrate and insulin, and higher intake of saturated fat¹⁶⁶. Some parents have referred to carbohydrate counting as a “double edged sword” because the greater dietary flexibility afforded by basal bolus regimens and insulin.

Interestingly, it is thought that carbohydrate counting is a much simpler approach to insulin dosing in practice because of the availability of carbohydrate contents for packaged foods. However in reality, even when presented packaged foods with their nutrition information label, only 23% of adolescents aged 12-18 years could correctly identify the carbohydrate content of the meal within 10 g of the true value, as discussed in the study above¹⁵⁹. In fact, the packaged foods with a label were among some of the least accurately assessed foods presented.

1.3.4.4 Summary

Carbohydrate counting is recommended to assist patients to match their insulin to flexible food choices, yet given the weaknesses in the theoretical basis for carbohydrate counting, alternate methods of determining prandial insulin dose should also be explored.

1.3.5 Warsaw Pump Therapy School Program

The Warsaw Pump Therapy School (WPTS) in Poland has proposed an alternative approach to traditional carbohydrate counting¹⁶⁸. This method was developed based on the premise that protein and fat, in addition to carbohydrate, increased insulin requirements and that the

macronutrient composition of the meal will affect the meal absorption pattern. Their method therefore, builds upon traditional carbohydrate counting by introducing a ‘Fat-Protein Unit’ (FPU) to determine supplementary mealtime insulin doses and emphasises the use of varying insulin delivery patterns using insulin pump therapy technology.

1.3.5.1 Definition

A FPU is defined as 100 kcal (418 kJ) of fat and/or protein ¹⁶⁸. The total number of FPUs in a meal are summed and multiplied by an individualised insulin ratio. Similar to traditional carbohydrate counting, the carbohydrate content in the meal is also counted as 10 g carbohydrate units (CU). The total prandial insulin dose for each CU and FPU is based on the individualised ICR.

Insulin doses are delivered in a dual wave insulin delivery pattern, meaning a proportion of the insulin is delivered immediately, while the remainder of the dose is delivered gradually over an elected period of time. The proportional split and the duration of the insulin delivery is determined by the number of FPUs of the meal. For a meal containing no FPU, the total insulin dose is delivered immediately, for 1 FPU this component of the dose is delivered over 3 h, extending to 4 h for 2 FPU, 5 h for 3 FPU and to 8 h for 3 or more FPU.

1.3.5.2 Clinical studies

The WPTS Program was shown to be effective for controlling postprandial glycaemia in one randomised, controlled clinical experimental trial¹⁶⁹. Twenty-six children with type 1 diabetes using insulin pump therapy, aged 12-18 yr, were randomised to one of two groups, Group A used traditional carbohydrate counting, while Group B had their mealtime insulin

dose determined using the WPTS Program method of carbohydrate + FPU counting. Both groups received the same pizza meal at dinnertime, consisting of 46.8 g of carbohydrate, 33.1 g of fat and 25.4 g of protein (ie 4.5 CU and 4.0 FPU). Group A received the insulin for the carbohydrate component of the meal only, delivered as a standard, immediate bolus. Group B received the insulin dose determined by the CUs and FPUs, delivered over 6 h. Postprandial blood glucose levels over 6 hs, were significantly lower in the Carbohydrate + FPU counters than in those counting carbohydrate alone, from 60 mins.

A cross-sectional study of 499 bolus records from children with type 1 diabetes using insulin pump therapy, aged 0-18 yr, were assessed for frequency of dual wave boluses¹⁶⁸. All of the patients included in the study had been taught to use dual-wave boluses with the carbohydrate + FPU counting. The study showed that those using 2 or more dual wave boluses each day achieved the lowest HbA1c values compared with those administering an average of less than 1 per day. Although this study is not direct evidence for the use of the WPTS program, it does assume that the application of a dual-wave bolus within this clinic population reflects compliance with this method of food counting and estimating insulin doses.

To assist in the calculation of insulin doses, a bolus and food calculator 'Diabetics' was developed by the WPTS¹⁷⁰. As expected with the introduction of technology, comparison of glycaemic control in children using the 'Diabetics' software, with those calculating their doses using calorie tables and mental calculations showed the software significantly reduced 2 h post-prandial blood glucose levels and glycaemic variability. Surprisingly, this did not translate to improvements in HbA1c or insulin doses.

1.3.5.3 Clinical considerations

The WPTS program builds on traditional carbohydrate counting by providing a practical prescription for estimating the insulin dose and delivery pattern required for meals containing protein and fat as well as carbohydrate. This approach reflects an important shift in clinical practice, as it is the first method described in the literature incorporating protein and fat into prandial insulin dosing in type 1 diabetes.

There are several important clinical advantages to this method. In addition to providing a quantitative approach to mealtime insulin dosing for protein and fat, the method also aims to mimic normal physiological insulin release patterns by utilising insulin delivery pattern options available with modern insulin pump technology. By better matching exogenous insulin doses with physiological demand, glycaemic control can potentially be improved.

Furthermore, the method can be easily integrated into clinical practice, as carbohydrate, protein and fat contents of foods are readily available on food packaging. One of the limitations of the WPTS program however, is the complexity of insulin dose calculations. Carbohydrate counting is often critiqued for the literacy and numeracy skills required to master the method in practice, however the WPTS method is far more complicated in practice. There are a number of calculation steps involved in calculating the total insulin dose and delivery and, although the macronutrient weights are available, the calories for protein and fat, which are required for the calculation of the number of FPU's, are not listed on food packaging.

In addition to this practical limitation, there are limitations in the theoretical basis of this method. The WPTS program assumes that all carbohydrate, protein and fats modulate insulin requirements uniformly. However, just as carbohydrate from different foods sources has been shown to produce wide variations in blood glucose and insulin responses, so to have fat and

protein from different food sources. Furthermore this method also does not account for the effect of nutrient interactions within foods. Furthermore, this method is limited to individuals using insulin pump therapy. Although insulin pumps are becoming increasingly popular, the majority of patients with type 1 diabetes rely on multiple daily insulin injections, which does not allow for dual-wave insulin release.

While some of the initial published trials suggest the WPTS program may be effective for optimising glycaemic control in type 1 diabetes, there is a paucity of evidence. At the time of this review, there have been no published randomised, controlled clinical trials comparing the WPTS Program method with another method of determining insulin dose such as carbohydrate counting in the real-world setting. The one experimental trial showed significant improvements in 2 h post-prandial glycaemia however these results are confounded by differences in insulin delivery patterns and without well-designed, randomised controlled trials, it has yet to be seen whether this translates to clinically meaningful improvements in long-term glycaemic control. Furthermore, studies have only been conducted in the paediatric population and thus the efficacy would also need to be established in the adult population.

The WPTS program integrates additional insulin for protein and fat with dual-wave bolusing. Since changes in the insulin delivery pattern could potentially improve glycaemic control, this element acts as a potential confounder in assessing the efficacy of this method of estimating the total insulin dose required for meals containing protein and fat.

1.3.5.4 Summary

In summary, the WPTS program offers an alternative to traditional carbohydrate counting by incorporating additional insulin dosing for the protein and fat contents of meals, however there are significant theoretical and practical limitations to the method.

1.3.6 Food Insulin Index

The Food Insulin Index (FII) is a novel algorithm of ranking foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food¹⁶. It was originally proposed as a tool in nutritional epidemiology to study the effect of diets calculated to have a high insulin demand on the risk of development of diabetes and adverse blood lipid profiles¹⁷¹. A possible role in the dietary management of type 1 diabetes was also suggested.

1.3.6.1 Definition

The FII is methodologically defined as the incremental area under the plasma insulin curve elicited by a 1000 kJ portion of a test food expressed as a percentage of the response to a 1000 kJ portion of the reference food (glucose) within one lean, healthy subject. The final FII of a food is calculated as the average FII in 10 subjects.

$$\text{Food Insulin Index (FII)} = \frac{120\text{min AUC}_{\text{Insulin}} \text{ for 1000 kJ test food}}{120\text{min AUC}_{\text{Insulin}} \text{ for 1000 kJ reference food}} \times 100$$

Using food energy as the constant allows all foods to be included, not just those containing carbohydrate, and thus all dietary components and their metabolic interactions can be considered, allowing a more holistic approach to determining insulin demand.

1.3.6.2 FII database

A systematic FII database has been established and the data for 121 foods, accumulated between 1995 and 2009 were routinely added to the database¹⁶. The tested foods included the top 100 sources of energy in the American diet, representing 10 food categories. The FII values of foods vary widely both within and between food groups. On a scale where the reference food 1000 kJ anhydrous glucose = 100, jellybeans produced the highest FII value (117 ± 12), while gin produced the lowest FII (1 ± 1). Common foods in the western diet, such as white bread (FII = 73) and potato (FII = 88), are among the most insulinogenic foods. Highly refined bakery products, snack foods and breakfast cereals induced substantially more insulin secretion per portion of food (gram-basis) than did the other foods tested. There were significant differences within food groups despite similarities in macronutrient composition. For example, dairy products ranged from FII = 18 for cream cheese to FII = 84 for low fat strawberry yoghurt, a 4.5-fold difference. Interestingly, protein-rich foods induced significant insulin responses, with some foods having FII values even higher than some carbohydrate-rich foods. Beef steak for example, contains no carbohydrate but has a FII value of 37, while an isoenergetic portion of grain bread contains 40 g of carbohydrate and a similar FII of 44.

Correlations between the FII, and the glycaemic load (GL), glycaemic index (GI), available carbohydrate, protein, fat, starch, sugar and fibre showed the GL was the most powerful predictor of postprandial insulinaemic response ($r = 0.68$, $p = 0.01$). Fat was also a significant

predictor ($r = -0.60$, $p = 0.03$)¹⁶. Although carbohydrate is widely considered the best predictor of insulinaemic responses to foods, it was not significantly correlated with insulin responses ($r = 0.53$, $p = 0.06$). Protein was also not significantly correlated ($r = -0.46$, $p = 0.12$).

1.3.6.3 Clinical studies

1.3.6.3.1 Healthy population

The efficacy of the FII for predicting insulin responses to realistic meals has been tested in healthy adults. Lean, healthy adults ($n = 10$ or 11 for each meal) consumed 13 different isoenergetic (2000 kJ) mixed meals of varying macronutrient content and their plasma insulin response was measured¹⁶. As anticipated, the observed insulin response varied over a wide range with significant differences between the meals ($P < 0.001$) but was best predicted by the calculated insulin demand of the meal (calculated from the pre-determined FII of the component foods relative to reference food white bread) ($r = 0.78$, $P = 0.0016$). This study confirmed that the database of single foods could be used to predict responses to mixed meals where the amount of energy from different foods and macronutrients varied. Surprisingly, in that context, carbohydrate, fibre and protein content were found to be relatively poor predictors of the overall insulin response, while GL and fat content were also significant predictors, but less so than the FII. In fact, mixed meals with similar carbohydrate content produced widely disparate insulin responses. For example, a meal containing beef steak and potatoes with ~40 g of carbohydrate produced twice the insulin response of a meal composed of grain bread, peanut butter and milk, despite a similar amount of carbohydrate (~37 g). This underscored the inherent difficulty with estimating insulin demand (or insulin dose in the case of type 1 diabetes) based on carbohydrate alone.

In a subsequent study, 10 healthy adults consumed a high and low FII diet (3 consecutive meals over 8 h) in a randomised, crossover design. The two iso-energetic diets were matched for macronutrients, fibre and GI but had a two-fold difference in predicted insulin demand (FII = 65 vs 30). The low FII diet reduced the mean insulin response (iAUC) by 53% compared with the high FII diet, despite no significant differences in glycaemic response.

These studies provided the first clinical evidence of the physiological validity of the concept of food insulin index as a measure of day-long glucose and insulin profiles. The findings implied that if the database were sufficiently large, the FII classification may have clinical application in estimating insulin demand of different meals and diets in type 1 diabetes.

1.3.6.3.2 Type 1 diabetes

At the start of this PhD project (July 2011), only one study exploring the utility of the FII for estimating insulin demand in type 1 diabetes had been undertaken. In that study¹⁸, 28 adults with type 1 diabetes using continuous subcutaneous insulin infusion therapy (CSII, insulin pump) consumed two different iso-energetic breakfast meals, matched for GI, fibre and calculated insulin demand (both FII = 60) but with a 2-fold difference in carbohydrate content (75 g vs 41 g). The meals were consumed in random order on 3 consecutive mornings after an overnight fast. Meal A was consumed once with the insulin dose determined by the carbohydrate content of the meal, while Meal B was consumed twice, once based on the carbohydrate content and once based on the predicted insulin demand. Since the two breakfast meals had the same FII, the same insulin dose was applied for Meal B as for Meal A when the dose was determined by the FII even though Meal B contained only half as much carbohydrate. Compared with carbohydrate counting, the FII algorithm significantly decreased glucose incremental area under the curve over 3 h (-52%, $p = 0.013$), peak glucose

excursion (-41%, $p = 0.01$) and improved the percentage of time within the normal blood glucose range (4-10 mmol/L) (+ 31%, $p = 0.001$). Although twice as much insulin was given for Meal B using the FII as for carbohydrate counting, there were no significant differences in the occurrence of hypoglycaemia. The study demonstrated that the FII based on physiological insulin demand in healthy adults may be a useful tool for estimating mealtime insulin dose in patients with type 1 diabetes.

1.3.4.4. Epidemiological studies

Hyperinsulinaemia has been identified as risk factors for several chronic diseases, including type 2 diabetes, cardiovascular disease and cancer. Dietary patterns characterised by a low insulin demand (ie low FII) may play a role in the prevention and treatment of these chronic diseases. Epidemiological studies have investigated the relationship between the FII and colorectal cancer¹⁷², prostate cancer¹⁷³, pancreatic cancer¹⁷⁴, however no significant association between a high FII diet and risk of these cancers was found. One study looking at the FII in relation to biomarkers of glycaemic control, plasma lipids and inflammation markers, found that those in the highest quintile of FII had 26% higher triglyceride concentrations than those in the lowest quintile ($P < 0.0001$)¹⁷¹. This association was strongest for obese subjects. HDL-cholesterol was also inversely associated with the FII in obese subjects. The FII was not significantly associated with fasting biomarkers of glycaemic control, inflammation or other plasma lipids including, plasma C-peptide, HbA1c, LDL cholesterol, CRP, or IL-6. Further work is warranted in this area with other diet-related chronic diseases and their biomarkers.

Puberty has also been associated with physiological insulin resistance and the potential for overweight development. One longitudinal study¹⁷⁵ in boys and girls before and after puberty found that a higher dietary FII and a higher food insulin load at the start of puberty were associated with higher levels of percentage of body fat in young adulthood but not body mass index (BMI, kg/m²), even after adjustment for early life, socioeconomic and nutritional factors. This suggested that postprandial increases in insulinaemia (ie a high FII diet) during puberty may have an unfavourable effect on the body composition in young adulthood.

1.3.6.4 Summary

In summary, early studies into the FII indicate it is a promising tool for improving the mealtime insulin dosing algorithm in type 1 diabetes. Further research is required to determine its clinical efficacy and the feasibility of its integration into clinical practice.

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CHAPTER 2

The efficacy of carbohydrate counting
in type 1 diabetes: a systematic review
& meta-analysis

2.1 Abstract

Objective: Although carbohydrate counting is the recommended dietary strategy for achieving glycaemic control in people with type 1 diabetes, the advice is based on narrative review and grading of the available evidence. Our objective was to conduct a systematic review and meta-analysis of the efficacy of carbohydrate counting on glycaemic control in adults and children with type 1 diabetes.

Methods: We screened and assessed randomised controlled trials (RCTs) of interventions >3 months duration that compared carbohydrate counting with general or alternate dietary advice in adults and children with type 1 diabetes. Change in glycated haemoglobin (HbA1c) was the primary outcome. Hypoglycaemia, insulin dose, fasting plasma glucose, weight change and quality of life were secondary outcome measures.

Results: Of 311 potentially relevant studies, we identified 7 eligible RCTs, comprising 599 adults and 104 children with type 1 diabetes. Study quality score averaged 7.6 out of 13. Overall there was no significant improvement in HbA1c with carbohydrate counting versus the control or usual care (-0.35%, 95% CI: -0.75-0.06; $p = 0.096$). We identified significant heterogeneity, potentially related to study design. In the five studies in adults using a parallel design, there was a -0.64% point improvement in HbA1c with carbohydrate counting (95% CI: -0.91 - -0.37; $p < 0.0001$). Six studies reported a non-significant decrease in the frequency of hypoglycaemia using carbohydrate counting.

Conclusions: There is modest evidence to recommend carbohydrate counting over alternate advice or 'usual care' in adults with type 1 diabetes. Additional studies are required to support carbohydrate counting over other methods of matching insulin dose to food intake.

Publication: Bell K, Barclay AW, Petocz P, Colagiuri S, Brand-Miller JC. The efficacy of carbohydrate counting in type 1 diabetes: a systematic review and meta-analysis. *Lancet Diabetes & Endocrinology* 2014; 2(2): 133-140 (**Appendix 1**).

2.2 Introduction

Type 1 diabetes is an autoimmune disorder characterised by chronic hyperglycaemia resulting from an absolute endogenous insulin deficiency. Current medical management revolves around exogenous insulin therapy to restore blood glucose levels to within an optimal range. At present there is no cure for type 1 diabetes, therefore effective strategies to assist in the achievement and maintenance of normoglycaemia are required to promote the acute and long-term health and wellbeing of individuals with type 1 diabetes.

Carbohydrate counting has long been considered the cornerstone of intensive insulin therapy, with bolus insulin doses matched to the total carbohydrate content of the meal.¹ This practice is based on the premise that carbohydrate is the predominant macronutrient contributing to the rise in post-prandial glycaemia. Carbohydrate counting ranges from an awareness of carbohydrate-containing foods and their impact on blood glucose levels through to counting the number of carbohydrate exchanges (15 g), portions (10 g) or grams of carbohydrate eaten. An insulin: carbohydrate is used to calculate the bolus dose required.²

Thirty years ago, Slama et al. showed a significant correlation between the amount of carbohydrate consumed and the dose of insulin needed to restore blood glucose levels using an artificial pancreas.³ Further work confirmed the linear relationship between carbohydrate intake and insulin requirement.⁴ However, the underlying theoretical basis and its practical utility have since been questioned.⁵⁻⁹ Bao et al., for example, showed that available carbohydrate could explain much of the variance in glucose response to iso-caloric portions of single foods, but was not a significant predictor of the response to mixed meals containing variable amounts of carbohydrate, fat and protein.¹⁰ Moreover, the same amount of available carbohydrate from different food sources is known to produce significantly varying blood glucose responses in both healthy and diabetic subjects.^{5,11,12} Indeed, the predictable

difference in responses to different carbohydrate-containing foods is the basis of the glycemic index (GI).

Surprisingly, the efficacy of carbohydrate counting has not been assessed as a benchmark for other dietary strategies. Current international recommendations supporting the use of carbohydrate counting in practice are based simply on narrative review and grading of the limited available evidence.^{1,8,13} Hence, our objective was to conduct a systematic review and meta-analysis of randomised controlled trials (RCT) comparing carbohydrate counting interventions with general or alternate dietary advice in adults and children with type 1 diabetes. Glycaemic control as judged by glycated haemoglobin (HbA1c) was the primary outcome measure.

2.3 Methods

The study was designed and reported in accordance with the PRISMA guidelines, an evidence-based, 27-item checklist for reporting systematic reviews and meta-analyses to ensure complete and transparent reporting.

2.3.1 Search strategy

We searched all relevant biomedical databases, including the Medical Literature Analysis and Retrieval System Online (MEDLINE), the Excerpta Medica (EMBASE), the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Thomson Reuters (formally ISI) Web of Knowledge and the Cochrane Central Register of Controlled Trials. In consultation with a medical librarian, we developed a search strategy based on an analysis of medical

subject headings, terms, and key text words from January 1980 to August 2013 (**Appendix 2**).

A start date of January 1980 was intentionally chosen as HbA1C assays were becoming routinely available in the early 1980s.¹⁴ We combined terms for “randomised controlled trials”, “type 1 diabetes”, “glycaemic control” and “carbohydrate counting”. Reference lists from relevant meta-analyses, systematic reviews, and clinical guidelines were also examined.

2.3.2 Study selection

Two review authors (KB and AB) independently screened all study titles and abstracts identified through the search strategies against the predetermined selection criteria described below, to identify potentially relevant studies. Duplicate records were removed and multiple papers on the same study were collated as one. Full-text copies of potentially relevant studies were sourced and independently assessed by the reviewers for compliance with the selection criteria. To be included, studies had to be written in English and published between January 1980 and August 2013. All published randomised and quasi-randomised controlled clinical trials of interventions that compared the management of type 1 diabetes with and without carbohydrate counting in either children or adults with type 1 diabetes for at least 3 months were included. RCTs in pregnant women were acceptable. ‘Carbohydrate counting’ included all methods of quantifying the amount carbohydrate consumed for the purpose of determining prandial insulin dose, as per the American Diabetes Association definition.¹ This included counting in grams or using carbohydrate exchanges or portions and both flexible and fixed insulin therapy. Interventions where insulin therapy or diabetes education was simultaneously intensified were accepted for inclusion because carbohydrate counting is intricately linked with insulin therapy. This factor was noted as a potential confounder.

2.3.3 Data extraction and quality assessment

The data from included studies were independently extracted using a predetermined form by two review authors (KB and AB) and compared for accuracy. Any differences between reviewers' data extraction results were resolved through discussion. Where there was uncertainty, authors were contacted for clarification.

The primary outcome measure was HbA1c. Secondary measures included 1) number overall and severity of hypoglycaemic episodes; 2) fasting plasma glucose; 3) insulin dose required to maintain glycaemic control; 4) body weight change and 5) quality of life (measured by a validated instrument).

The quality of each study was independently evaluated by two review authors (KB and AB) based on the following factors and was scored according to the corresponding criterion (**Table 2.1**). Quality assessment items included 1) random sequence generation; 2) allocation concealment; 3) blinding of outcome assessment; 4) subject attrition rate or 'lost-to-follow-up' rate; 5) incomplete outcome data; 6) protocol deviation 7) selective reporting and 8) use of an attention placebo in the control group.

Table 2.1: ‘Assessment of risk of bias’ scoring criterion.

Bias Type	Bias Source	Criteria	
Selection Bias	Random sequence generation	0 - Unclear/Not randomised 1 - Pseudo-randomised 2 - Appropriately randomised	
	Allocation concealment	0 - Not concealed/Unclear 1 - Concealed	
Detection Bias	Blinding of outcome assessment	0 - Unclear/Not blinded 1 - Outcome assessor blinded	
Attrition Bias	Participant attrition rate or ‘lost-to-follow-up’ rate	0 - $\geq 25\%$ 1 - 15 - 24.9% 2 - 5.0 - 14.9% 3 - 0 - 4.9%	
		Incomplete outcome data	0 - Not discussed 1 - Used 1 method of dealing with missing data and discussed potential impact 2 - Compared multiple strategies for dealing with missing data and discussed impact on results/conclusions.
			Protocol deviation
Reporting Bias	Selective reporting	0 - Outcome measure(s) not reported with no explanation 1 - Outcome measure(s) not reported with explanation/discussion of impact 2 - No outcome measure(s) omitted	
Other Bias	Attention Placebo	0 - No attention placebo 1 - Attention placebo	

2.3.4 Statistical analysis

The Comprehensive Meta-Analysis (CMA) package¹⁵ was used to analyse the data. As all data collected were continuous, the results were expressed as the difference in means calculated from end of treatment values, with 95% confidence intervals. Cross-over studies were included and analysed using the mean and standard deviation (SD) of the change from the baseline to endpoint of each intervention time period. Heterogeneity between studies was assessed using the Chi-squared test, with a significance level of 0.05 considered evidence of heterogeneity. Funnel plots were used in exploratory data analyses to assess for the potential existence of publication bias. The results of clinically and statistically homogenous studies were pooled and meta-analysed using the random-effects model to provide estimates of the efficacy of carbohydrate counting. Statistical significance was set at a p value of < 0.05 for all outcome measures.

The following subgroup analyses were performed, 1) carbohydrate counting method (grams versus 10 g portions versus 15 g exchanges); 2) adults versus children; 3) pregnant versus not pregnant.

Sensitivity analyses were performed by using the ‘one study removed’ sensitivity analysis and by excluding studies identified as having a high risk of bias.

2.4 Results

2.3.1 Study selection

From the 311 studies identified through the literature search and additional hand-searching, 18 papers were identified as potentially relevant,¹⁶⁻³³ (**FIG 2.1**). Of those 18 studies, 8 were excluded because there was no control group (or no control group without carbohydrate counting),¹⁶⁻²³ one was not an original article,²⁴ one was in type 2 diabetes²⁵ and one did not report HbA1c values²⁶. The remaining seven studies,²⁷⁻³³ totaling 703 subjects (599 adults, 104 children), met the inclusion criteria. The subject characteristics and outcomes of these studies, including the number and mean age of subjects, study design, intervention duration and study quality score, are shown in **Table 2.2**.

Six studies were conducted in parallel^{27,28,30,31,33} and one was a cross-over with 3 interventions.²⁹ Six of the seven studies were conducted in adults^{27,29-33} and one recruited children aged 8 -13 years²⁸. All studies were conducted in an outpatient clinical setting, with three providing individual appointments²⁸⁻³⁰ and four conducting group education sessions^{27,31,33}. Study duration averaged 11 months (range 3.5 to 30 months). Each study used carbohydrate intake to determine insulin dose, although a variety of different methods of quantifying carbohydrate counting were taught. Two studies instructed subjects to count in grams of carbohydrate^{29,30}, one study used 10 g carbohydrate exchanges,²⁷ another used 15 g carbohydrate exchanges²⁸ and three studies^{31,33} did not specify how carbohydrate was quantified and the authors could not be contacted. In six of the seven studies, subjects in the control groups received 'usual care' and general nutrition education.^{27,29-31,33} One study compared carbohydrate counting with a flexible (non-measured) low GI diet.²⁸

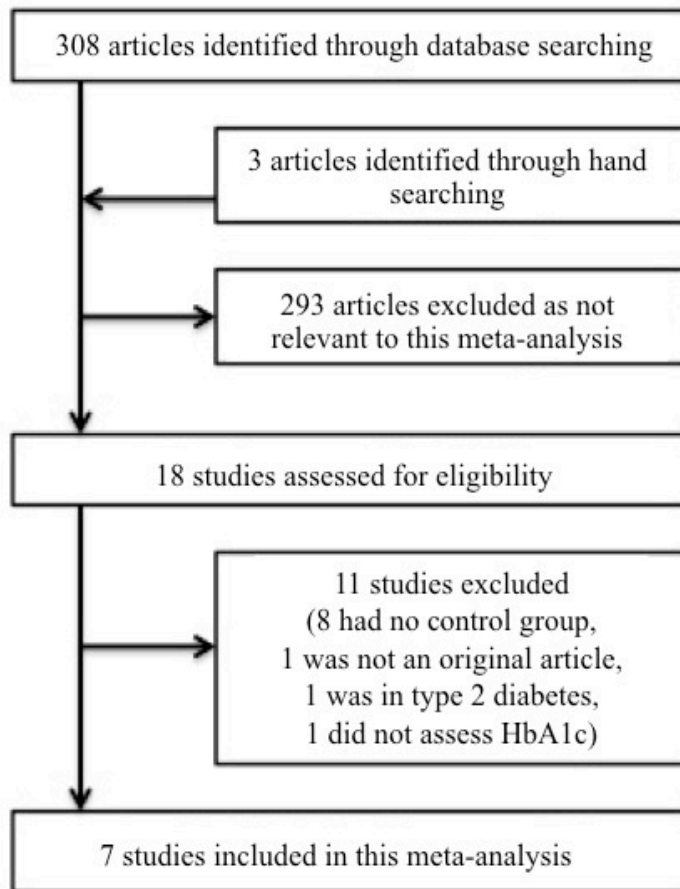


Figure 2.1: Flowchart of Study Selection

Assessment of the quality of the studies revealed an average score of 7.6 out of a possible 13 points (range: 5-10). All studies used appropriate randomisation strategies; however none of the studies had adequate intervention allocation concealment and none blinded the outcome assessor. The highest scoring studies had the lowest attrition rates and demonstrated the least risk of bias. Four of the six studies had concurrent intensification of the insulin regimen,^{27,29,30,33} and only four provided an attention placebo i.e. subjects in the control group had equivalent amounts of contact time as those in the carbohydrate counting group.^{28,29,32,33}

Table 2.2: Summary of included studies

Study	Study Design	Study Length	Number of Subjects (Total sample size (No. completing study/ No. allocated, attrition rate))	Population (Diabetes type, Gender, Age, Country Conducted)	Intervention (Description, Education Mode, Carbohydrate Counting Method)	Control (Description, Education Mode, Carbohydrate Counting Method)	HbA1c (Baseline Mean \pm SD – Endpoint Mean \pm SD, Change Mean \pm SD. P value between groups, where available)	Secondary Outcomes (Baseline Mean \pm SD – Endpoint Mean \pm SD, Change Mean \pm SD. P value between groups, where available)	Intention to Treat Analysis?	Quality Score (max. 13)
DAFNE 2002 ²⁷	RCT	12 months	169 (I: 68/84, 19%, C: 72/85, 15%)	type 1 diabetes; 44% male; mean age 40 \pm 9 years; England	5 day course run by diabetes educator in matching insulin to carbohydrate intake; group education; 10 carbohydrate exchanges	Usual care	HbA1c (%) I: 9.4 \pm 1.2 – 8.4 \pm 1.2, C: 9.3 \pm 1.1 – 9.4 \pm 1.1, $p < 0.0001$	Weight (kg) I: 80.5 \pm 16.7 – 81.5 \pm 16.9, C: 77.4 \pm 13.4 – 77.3 \pm 13.4, $p = 0.11$ Hypo (% subjects experiencing severe hypo in past 6 months; coma or requiring third party assistance) I: 22-18, C: 11-15, $p = 0.67$ Insulin Dose (U/kg) I: 0.71 – 0.74, $P = 0.017$, C: 0.71 – 0.70, $p = 0.47$ QoL (ADDQoL) I: -2.0 \pm 1.6 – -1.6 \pm 1.6, C: -1.9 \pm 1.3 – -1.9 \pm 1.4, $p < 0.01$	No	9

Gilberts on et al. 2001 ²⁸	RCT	12 months	104 (I: 38/49, 22%, C: 51/55, 7%)	type 1 diabetes; 51% male; mean age I: 10 ± 2 years; C: 11 ± 2 years, Australia	Meal plan with set number of carbohydrate exchanges per meal/snack; individual education - 1 session with dietitian; 15 g carbohydrate exchanges	Low glycaemic index diet with no portion prescription but general guide; Individual education - 1 individual session with dietitian	HbA1c (%) I: 8.6 ± 1.4 – 8.6 ± 1.4, C: 8.3 ± 1.3 – 8.0 ± 1.0, p = 0.05	Weight Measured but not reported. Hypo (Mean number of episodes/month; <3.5mmol/L) I: 7.3 ± 5.7 – 5.8 ± 5.5, C: 6.9 ± 6.2 – 6.9 ± 6.8, p = 0.37 Insulin Dose (U/kg) I: 0.9 ± 0.3 – 1.00 ± 0.3, C: 1.0 ± 0.3 – 1.1 ± 0.3, p = 0.87	Ye
Kalergis et al. 2000 ²⁹	RCT – cross- over	3.5 months	21 (I: 15/21, 29%, C: 15/21, 29%)	type 1 diabetes; 40% male; mean age I: 38 years; C: 38 years, Canada	Meal plan with insulin adjusted using 1 unit:10 g ratio; Individual education - monthly clinic visits and then weekly telephone; gram increments	Meal plan using food group exchange system and no insulin adjustments; Individual education - monthly clinic visits and then weekly telephone	HbA1c (%) I: 7.6 ± 1.3 – 7.2 ± 0.9, -0.21 ± 0.18, C: 7.7 ± 1.24 – 7.9 ± 1.4, -0.24 ± 0.22, NS	Weight (kg) I: 67.7 ± 3.0 – 67.4 ± 3.2, C: 67.7 ± 3.0 – 68.2 ± 2.9 Hypo (episodes per 100 patient years at endpoint; < 4mmol/L)) I: 53. C: 48, NS QoL (DQOL) I: 2.0 ± 0.10 – 1.8 ± 0.11, C: 2.0 ± 0.10 – 2.0 ± 0.13, NS	Ne

Laurenz i et al. 2011 ³⁰	RCT	24 weeks	61 (I: 28/30, 7%, C: 28/31, 10%)	type 1 diabetes; 54% male; mean age I: 41 ± 10 years; C: 40 ± 10 years, Italy	Education programme including estimating grams of carb and matching with insulin; Individual education - 4-5 visits with dietitian in 12 weeks; gram increments	Usual care	HbA1c (%) I: 7.9 ± 0.9 (baseline), - 0.4 (mean change), C: 8.10 ± 1.5 (baseline), - 0.05 (mean change), p = 0.05	Insulin Dose (U/day) I: 36 (median), C: 33 (median), p = 0.282	Not stated
Scavone et al. 2010 ³¹	RCT	9 months	256 (I: 73/100, 27%, C: 156/156, 0%)	type 1 diabetes; 49% male; mean age I: 39 ± 11 years; C: 39 ± 11 years; Italy	Nutrition education programme including estimating carbohydrate content and matching insulin to carbohydrate; Group education - 1 session/wk for 4 weeks then 3 monthly reviews to 9 months; Not stated.	Usual Care	HbA1c (%) I: 7.8 ± 1.3 – 7.4 ± 0.9, C: 7.5 ± 0.8 – 7.5 ± 1.1, p <0.01	Hypo (number of hypo events; <3.9mmol/L) I: 4, C: 7, p < 0.05 Insulin Dose (U/24 h) I: 23.5 ± 10.9. C: 27.7 ± 17.1, p = 0.03	Not stated

Schmidt et al. 2012 ³²	RCT	16 weeks	36 (I: 21/27, 22%, C: 8/9, 11%)	type 1 diabetes, I: 52% male, C: 75% male; mean age I: 41±10 years; C: 46±9 years; Denmark	Diabetes education program plus carbohydrate counting using individualised Insulin: carbohydrate ratio; Group education – 1 x 3hr session, 1 individual review, 2x 15min phone reviews; Not stated.	Diabetes education program, including empirical mealtime insulin adjustment based on prescribed doses.	HbA1c (%) I: 9.2 ± 0.6 – 8.4 ± 0.9, -0.8, C: 9.1 ± 0.7 – 8.9 ± 1.1, -0.1, p = 0.175	Hypo (Perceived frequency. Scored 0–6. Higher scores indicate higher perceived frequency) I: 2.3 ± 1.4 – 2.2 ± 1.1, -0.1, NS; C: 2.4 ± 1.3 – 1.8 ± 1.4, -0.6, NS; NS between groups. (Total reported episodes requiring third party assistance) I: 2, C: 1. Insulin Dose (U/kg/24 h) I: 0.7 ± 0.17 (baseline), -0.03 ± 0.11 (change mean); C: 0.7 ± 0.2 (baseline), -0.01 ± 0.07 (change mean). QoL (ADDQoL) I: -2.0 ± 1.7 – -1.8 ± 1.6, NS; C: -2.0 ± 1.7 – -1.4 ± 0.9, NS; NS between groups.	Yes
Trento et al. 2011 ³³	RCT	30 months	56 (I:27/27, 0%, C: 29/29, 0%)	type 1 diabetes; 67% male; mean age I: 37 ± 13 years; C: 37 ± 8 years, Italy	Carbohydrate counting program, including matching insulin to carbohydrate embedded into usual group care program; Group education - at least 8 education sessions; Not stated	Usual diabetes care program; group education - at least 8 education sessions	HbA1c (%) I: 7.6 ± 1.3 – 7.2 ± 0.9, -0.21 ± 0.18 NS, C: 7.7 ± 1.24 – 7.9 ± 1.4, -0.24 ± 0.22 NS within group, p < 0.05 between groups.	Weight (kg) I: 71.9 ± 10.0 – 71.9 ± 9.3, 0.65 ± 0.91, NS, C: 66.9 ± 10.7 – 67.1 ± 10.2, -0.17 ± 0.87, NS Hypo (Absolute number of severe hypo for group; requiring third party assistance) I: 5, C: 6 Insulin Dose (U/kg) I: 47.9 ± 10.6 – 46.1 ± 12.7, 0.82 ± 1.57, NS, C: 45.7 ± 12.6 – 49.3 ± 17.2, -4.4 ± 2.48, NS between groups. QoL (DQoL) I: 88.7 ± 9.2 – 78.0 ± 9.9, 10.7 ± 1.3, P < 0.0001, C: 88.7 ± 12.5 – 80.4 ± 11.7, 8.3 ± 1.47, p < 0.0001 within group, NS between groups	Yes

2.3.2 Carbohydrate counting and HbA1c

Meta-analysis of changes in HbA1c showed heterogeneous findings. Five studies favoured carbohydrate counting,^{27,30,31,33} while two favoured the control group.^{28,29} The change in HbA1c between baseline and end of the treatment ranged from -1.0% to +0.14% points in the carbohydrate counting groups, and -0.3% to +0.24% points in the controls. There was no significant improvement in HbA1c with carbohydrate counting versus the control or usual care, using the random effects model (-0.35%; 95% CI: -0.75-0.06; $p = 0.096$; **FIG 2.2A**). Assessment of the funnel plot showed no indication of asymmetry and thus there is no evidence of publication bias. Heterogeneity (Chi^2) between the studies was significant (32%, $p < 0.0001$), potentially related to study design. In the five studies that utilised a parallel design comparing carbohydrate counting with alternative advice or 'usual care', the difference in HbA1c between baseline and the end of the intervention was -0.64% points favouring carbohydrate counting (95% CI: -0.91 - -0.37; $p < 0.0001$; **FIG 2.2B**).

Certain assumptions were made to conduct the meta-analysis. The SD for the change in HbA1c from baseline to the end of the intervention is required to assess the significance of the change; however Trento et al.³³ was the only paper reporting this information. In Laurenzi et al.³⁰, the SD of the change was based on the reported p value for the difference between the intervention and control groups. The calculated SD was 0.45 for carbohydrate counting and 0.8 for the control group. Kalergis et al.²⁹ reported the SE of the change, although the values appeared too large and was more likely the SD of the change and was treated accordingly. Finally, for the remaining studies that did not report the SD of the change,^{27,28,31} a correlation of 0.5 between baseline and endpoint in the control data, and 0.6 in the intervention data was assumed, based on the values published in Trento et al.³³

As part of the sensitivity analysis and to verify the effects of these assumptions, the ‘one-study-removed’ analysis was conducted. All results remained consistent with only the removal of the studies by Gilbertson et al.²⁸ or Kalergis et al.²⁹ making the result significant ($p = 0.016$ and 0.015 respectively).

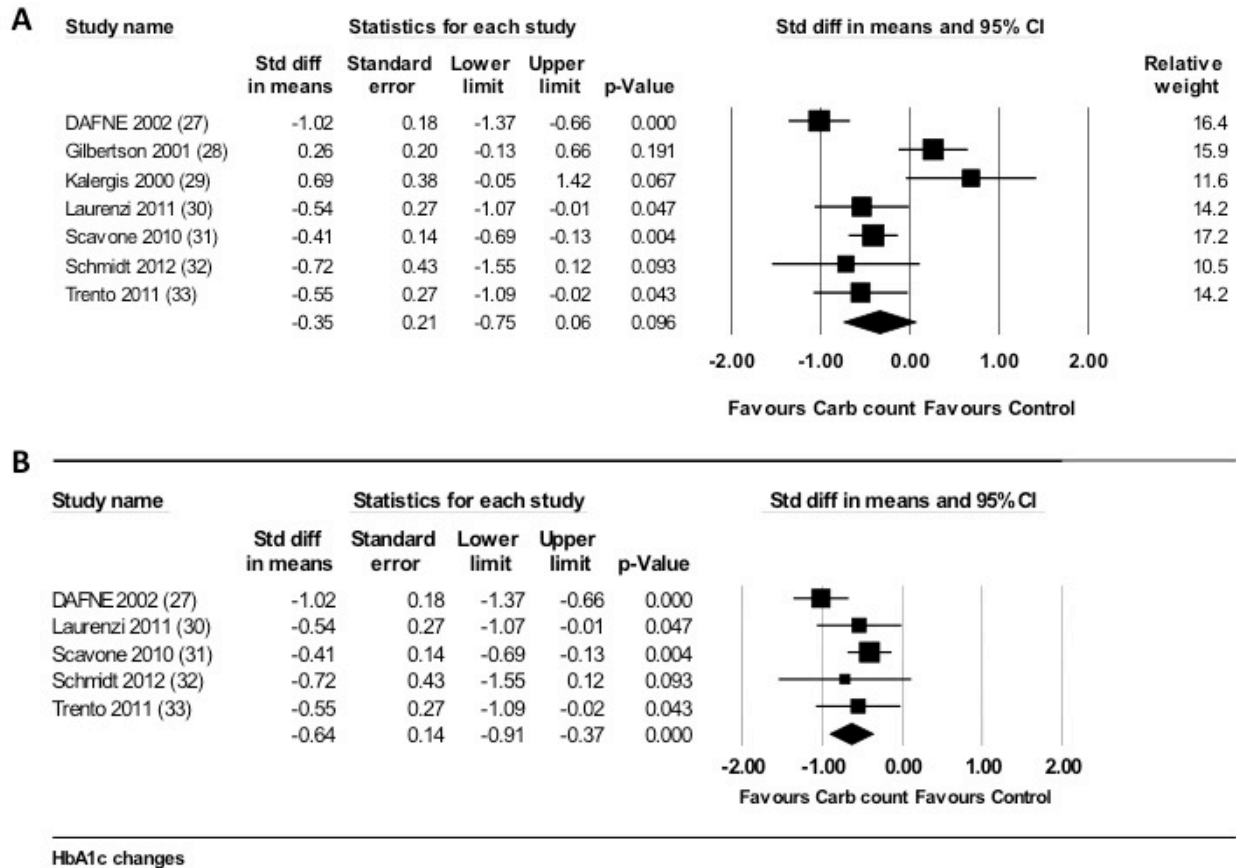


Figure 2.2: Meta-analysis of changes in glycated haemoglobin (HbA1c) after carbohydrate counting vs alternate advice or usual care in type 1 diabetes. All seven studies (A) studies and the five studies in adults employing a parallel design (B). The studies included are described in **Table 2.2**.

2.3.3 Carbohydrate counting and other indicators of glycaemic control

Hypoglycaemic events

There was inconsistency in the way hypoglycaemia was defined and reported. Four studies defined hypoglycaemia as an objective blood glucose value but the cut-off value varied from <4 mmol/L to <2.8 mmol/L.²⁸⁻³¹ Three studies captured ‘severe hypoglycaemia’ only, defined as whether third party assistance was required.^{27,32,33} Schmidt et measured perceived hypoglycaemia using a questionnaire³². Six studies reported hypoglycaemic events at the conclusion of the intervention, yet only three reported events at baseline.^{27,28,32} Two studies reported significantly less self-reported hypoglycaemia in the carbohydrate counting group, but did not report the level of significance,^{31,32}. In one study, there were two episodes of severe hypoglycaemia in the carbohydrate counting group compared with one in the control group.³² Overall, six studies reported a non-significant decrease in the frequency of hypoglycaemic events in the carbohydrate counting group.²⁷⁻³²

Quality of life

Five studies measured quality of life using a validated instrument,^{27,29,30,32,33} however only two studies used the same questionnaire and thus a meta-analysis was not possible. All five studies showed trends towards improved quality of life using carbohydrate counting, but only the ‘Dose Adjustment for Normal Eating’ (DAFNE) study group²⁷ showed a significant difference between groups. Laurenzi et al.³⁰ reported a significant improvement in scores relating to dietary restrictions ($p = 0.008$) but no other significant results and did not report the overall scores and associated p value.

Insulin dose

All studies reported the insulin dose as units of insulin/day or units of insulin/kg/day. Four out of the five studies that assessed changes in insulin dose reported a non-significant difference between groups with no consistent trends.^{27,28,32,33} Only Scavone et al.³¹ reported a significant difference between endpoint insulin doses (23 vs 28 units of insulin/24 h in the intervention vs control group respectively, $p = 0.03$) but did not report baseline or change values.

Body weight

Five studies measured weight at baseline and the end of the intervention^{27,28,29,32,33}, but 1 did not report the results²⁸. The four other studies all reported a non-significant difference between groups.

Fasting glucose

Only one study reported the change in fasting plasma glucose between baseline and the end of the 30-month study.³³ Fasting plasma glucose levels dropped non-significantly by 0.27 ± 1.50 mmol/L using carbohydrate counting.

Other sub-group analyses

Adults may be more accurate than children in estimating carbohydrate content of meals. In the 6 studies comprising of 599 adults^{27,29-33} there was a -0.4% difference in HbA1c favouring

carbohydrate counting (marginally significant $p = 0.048$). Only one study involved children²⁸ and therefore a comparison of adults vs children was not appropriate. There were too few studies to allow meta-analysis of different strategies used for quantifying carbohydrate (eg counting in grams vs 10 g or 15 g exchanges), and there were no studies in pregnancy.

2.5 Discussion

Our systematic review found seven eligible RCTs comparing the efficacy of carbohydrate counting on glycaemic control as determined by changes in HbA1c in adults and children with type 1 diabetes.²⁷⁻³³ The results were heterogeneous with five studies supporting carbohydrate counting and two trials (one crossover study, one in children) suggesting less quantitative methods were superior or equally effective. Overall, the difference was not statistically significant. However, in the five studies confined to adults, the difference in HbA1c favoured carbohydrate counting (-0.6% points), a result that was both clinically and statistically significant ($p < 0.0001$).

This systematic review and meta-analysis has several strengths. Although a number of reviews of carbohydrate counting have been published, to our knowledge, this is the first meta-analysis. Meta-analysis provides the opportunity to quantify the improvement in HbA1c that can be expected with the introduction of carbohydrate counting education. It also allows carbohydrate counting to be compared with other available glycaemic control strategies and offers a benchmark for future interventions. Our inclusion criteria ensured that only quality studies were eligible. We accepted only RCTs >3 months duration and independently assessed the risk of bias for each study and took this score into account when analysing and interpreting the findings. No study was found to have a high risk of bias and therefore no

studies were excluded for this reason. Additionally, our 'one study removed' sensitivity analysis, allowed us to examine whether lower quality scoring studies influenced the final result, however excluding the lowest scoring study did not alter the final result.

There are several limitations. Because so few studies met the inclusion criteria, caution must be applied in interpreting the results of this meta-analysis. Several studies from the Diabetes Teaching and Treatment Program (DTTP) and DAFNE program have shown improvements in HbA1c using carbohydrate counting,³⁴⁻³⁶ but were not designed as RCTs and lacked control groups. The limited number of included studies meant that most planned subgroup analyses could not be performed, including studies in children vs adults. The one study in children showed a significant difference between interventions, favouring the alternative approach (a low GI diet) over carbohydrate counting. Conversely, the observed trend to improved glycaemic control among adults should also be interpreted with care as most of the studies had a concurrent intensification of the insulin regimen in the intervention group, which may have reduced HbA1c independently of dietary advice. Similarly, three of the seven studies failed to provide an attention placebo for the control group.^{27,30,31} Since it is possible for glycaemic control to improve due to increased contact time with healthcare professionals, this may be an additional source of bias and needs to be taken into account when interpreting the results.

An additional limitation is that the included studies have not measured or reported compliance with the intervention nor the effectiveness of the education provided. The effectiveness of carbohydrate counting may ultimately be limited by both compliance and the ability of adults and children to accurately estimate carbohydrate content. Mehta et al.²³ showed that greater accuracy and precision in parent's ability to count carbohydrates was associated with a lower HbA1c in their child; however only precision was a significant predictor of HbA1c ($p = 0.9$

and $p = 0.02$ respectively). HbA1c was 0.8% points lower for parents who were >75th percentile for precision. The literature shows a wide variation in this ability, with some studies showing most participants are able to accurately estimate carbohydrate to within 10-15 g^{37,38} or within 15-20% of the true value,^{6,23} while other reports revealed only half could accurately estimate carbohydrate content³⁹ or had large variations in estimations⁴⁰.

Unfortunately, in the present study, the planned subgroup analysis comparing methods of quantifying carbohydrate could not be conducted because of the small number of eligible trials. Future studies should therefore investigate the role of precision and the effectiveness of one strategy over another.

In addition, the lack of consistency in way hypoglycaemia was measured and reported prevented the results from being meta-analysed. The data suggests a trend towards a decreased risk of hypoglycaemia with carbohydrate counting, indicating the reductions in HbA1c are a result of stabilised glycaemic control rather than just an overall lowering of blood glucose levels. It should be noted that reports of hypoglycaemic episodes based on blood glucose meter readings may not reflect the true incidence of hypoglycaemia, as some meters are not accurate below the normal range.

A prescribed meal plan may lower HbA1c irrespective of whether it includes carbohydrate counting. For example, Mehta et al.⁴¹ showed that HbA1c correlated with dietary adherence in youths aged 9 - 14 years, with those in the lowest tertile for dietary adherence having HbA1c 0.6 - 0.9% points higher than those in the higher tertiles. In the DCCT, HbA1c was 0.9% points lower in those following a prescribed diet 'most of the time' compared with those following a plan 'less than half of the time'.⁴² Carbohydrate counting with flexible insulin therapy may be advantageous to quality of life however, as it allows more dietary flexibility.

In practice, many people with type 1 diabetes have difficulty managing their postprandial

blood glucose levels. Ahola et al.⁴⁰ reported that only a third could maintain postprandial normoglycaemia, and even in those with apparently good metabolic control, ~40% experienced frequent hyperglycaemia. Given the weaknesses in the theoretical basis for carbohydrate counting, alternate methods of determining prandial insulin dose should also be explored. With developments in medical technology and a shift towards patient-centered care, insulin therapy has become more flexible and concomitantly medical nutrition therapy. Carbohydrate counting is recommended to match insulin doses to food choices yet alternate methods other than carbohydrate counting have rarely been studied. It is possible that other methods of matching insulin with food are not being studied because of the belief that carbohydrate counting is well-founded, evidence-based therapy. Indeed, this meta-analysis indicates how little high level evidence exists. Four studies suggest carbohydrate counting is better than 'usual care', but there are too few studies comparing carbohydrate counting with similarly intensive but different methods of matching insulin to food.

In summary, using systematic review and meta-analysis, we found seven RCT of at least 3 months duration comparing carbohydrate counting with usual care or alternate dietary advice for individuals with type 1 diabetes. Overall, there was no significant effect of carbohydrate counting (0.35%, $p = 0.096$), but a sub-analysis of five studies employing a parallel study design showed a -0.64% point improvement in HbA1c favouring carbohydrate counting ($p < 0.0001$). Further studies are needed, particularly in children and adolescents, to support the use of carbohydrate counting over other methods of matching insulin dose to food intake.

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CHAPTER 3

Testing of foods & beverages for their
Food Insulin Index and the systematic
evaluation of the associations between
postprandial glycaemia and insulinaemia
and nutritional content

3.1 Abstract

Objective: Dietary patterns which induce excessive insulin secretion may contribute to worsening insulin resistance and beta-cell dysfunction in susceptible individuals^{1,2}. This study had 3 aims: 1) test 26 common foods for their Food Insulin Index (FII) and Glucose Score (GS), 2) systematically evaluate the relationships between different dietary factors and postprandial physiological responses using the updated FII database and 3) validate previously generated equations for predicting FII and develop new predictive models.

Methods: Healthy subjects consumed 1000 kJ portions of 26 common single foods. Glycaemia and insulinaemia were quantified as area under the curve and the FII and GS calculated relative to an isoenergetic reference food. Simple and multiple linear regression analysis was used to evaluate correlations and develop improved predictive models.

Results: There were large differences in the GS and FII both within and between food groups for the 147 foods. GL, GI and available carbohydrate content were the strongest predictors of the FII, explaining 55%, 51% and 47% of variation respectively. Fat, protein and sugar were all also significant but relatively weak predictors, accounting for only 31%, 7% and 13% of the variation respectively. In the hierarchy of insulinotropic macronutrients, carbohydrate was the strongest predictor, followed by protein and then fat. Together the actual GS, sugar, protein and fat could be used to predict the final average FII value, explaining 78% of the variation. However, when only the nutritional composition of the food was considered, the equation explained about just half of the variability.

Conclusions: Macronutrient composition alone is a poor predictor of the FII, suggesting that *in vivo* testing is required to generate either the GS or FII itself. The postprandial insulin response to a

single food represents multiple nutritional and metabolic interactions that are difficult to predict with the precision needed for clinical application.

Acknowledgement: The existing FII database used in this chapter was collected progressively over a 15 yr period by a number of different individuals, including Dr. Susanne Holt, Fiona Atkinson, Dr Jiansong Bao, Vanessa de Jong, Kaniz Fatima, Karola Stockman, Laura Sampson, Mary Franz. Masters of Nutrition and Dietetic students, Sally Lane and Aleksandra Grasar, provided assistance with the practical work for the assessment of the Food Insulin Index values for some of the new foods presented in the chapter. See **Appendix 3** for the original FII database (121 foods).

3.2 Introduction

Metabolic health is of paramount importance in the prevention and management of chronic disease, including type 2 diabetes, overweight and obesity, cardiovascular disease and some types of cancers. As detailed in Chapter 1 (Section 1.3.4.4) dietary patterns which induce excessive insulin secretion are thought to contribute to worsening insulin resistance and beta-cell dysfunction^{1, 2}.

Carbohydrate has been identified as the sole macronutrient that *directly* increases postprandial blood glucose levels, and is thus the main dietary determinant of postprandial insulin secretion. However, numerous studies in healthy subjects have demonstrated that the same amount of carbohydrate from different food sources produces wide variations in glycaemic and insulin responses³⁻⁶. Furthermore postprandial insulin responses to typical mixed meals containing carbohydrate, protein and fat are not closely correlated with the carbohydrate content⁷, implying that carbohydrate counting will fall short in the management of type 1 diabetes. Indeed, the insulinotropic effects of protein and fat are well documented⁸⁻¹¹. While fat and protein have a modest effect on postprandial blood glucose levels (via increased hepatic glucose output and acute insulin resistance), they elicit significant insulin responses, in some cases comparable to that of carbohydrate¹²⁻¹⁷.

A comprehensive understanding of the relationship between dietary factors and physiological insulin secretion evoked by foods is required to inform the dietary management and prevention of chronic diseases. The glycemic index (GI) has proven a valuable tool for ranking carbohydrate foods on the basis of their postprandial blood glucose response relative to a reference food matched for carbohydrate content. The glycemic load (GL) is a measure of the potency of a carbohydrate food on glycemia and is calculated using the GI and the grams of carbohydrate in the serve size. However, the GI and GL concepts do not consider concurrent insulin responses, nor foods

containing little to no carbohydrate.

In order to systematically rate postprandial insulin responses for a broad range of common foods, a Food Insulin Index (FII) has been proposed which ranks foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food¹⁶. Using food energy as the constant allows all foods to be included, not just those with sufficient carbohydrate content, and thus all dietary components and their metabolic interactions can be considered, allowing a more holistic approach to determining insulin 'demand'. Additionally, a Glucose Score (GS) can be calculated using a similar equation but substituting the blood glucose response for the insulin response to determine the corresponding glycemic impact of the food. To date, 121 common foods in the western diet have been tested for their FII, but more research is needed to expand the database to represent the spectrum of foods consumed in the typical Australian diet and to increase our understanding of the relationship between dietary factors and postprandial insulin secretion. FII prediction equations have been published, although these were developed on the basis of just 38 test foods available at the time and their accuracy has not been validated¹⁸. The present study therefore aims to:

- 1) Determine the FII and GS for an additional 26 commonly-consumed single foods
- 2) Systematically evaluate the degree of association between different dietary factors and the postprandial physiological glycaemic and insulinaemic responses in healthy subjects consuming isoenergetic portions of 147 single foods
- 3) Validate previously generated regression equations for predicting FII and develop new predictive models using the updated FII database.

3.3 Methods

3.3.1 Study design

Healthy subjects (n = 10-11 for each test food) were recruited. Subjects were included if they were aged 18–65 years non-smoking, with a stable body weight and BMI between 19 and 25 kg/m².

Those with impaired fasting glucose or impaired glucose tolerance or food allergies, intolerances or eating disorders were excluded. The protocol was approved by the University of Sydney Human Research Ethics Committee and subjects gave informed consent (See **Appendices 4 – 7** for participant forms and questionnaires).

On the day prior to testing, subjects were instructed to refrain from unusual physical activity, avoid alcohol and legumes and to eat a high-carbohydrate, low-fat dinner meal, to avoid the ‘second meal effect’¹⁹ and other influences on glycaemia the following morning. On the morning of each testing session, subjects presented to the metabolic kitchen at the University of Sydney after a 10-12 h overnight fast. On separate occasions, each subject consumed a 1000 kJ portion of one of 26 test foods or the reference food (1000 kJ glucose powder dissolved in 250 mL of water) with 250 mL of water in random order. Due to their low energy density, the 6 non-starchy vegetables were tested in 300 kJ portions with an isoenergetic reference food. The test foods were chosen to represent a cross-section of commonly consumed foods in the Western diet that had not already been tested. The nutrition information for the tested foods is presented in Table 3.1. The subjects remained seated throughout the test session and were not permitted to eat or drink until the end of the session.

Fasting capillary blood samples were taken at -5 and 0 minutes and then postprandially at 15, 30, 45, 60, 90 and 120 minutes. Plasma glucose was analysed with the glucose hexokinase enzymatic assay on a centrifugal analyzer (model HITACHI 912; Hitachi, Tokyo, Japan). Plasma insulin was measured with an antibody-coated tube radioimmunoassay (Diagnostic Products Corporation, Los

Angeles, CA).

3.3.2 Statistical analysis

The incremental area under the 120 min curve (iAUC) was calculated using the trapezoidal method for both the glucose and insulin response for each test subject for each food. All subjects tested the reference food on 3 separate occasions, with the average metabolic response used as the basis for comparison with all other foods and thereby accounting for differences between subjects. The ISO standard for GI testing of intra-individual CV < 35% for the reference food was used.

The FII for each subject was determined as the iAUC of the insulin response elicited by the 1000 kJ portion of the test food expressed as a percentage of the average iAUC response to the 1000 kJ portion of the reference food (glucose). The final FII of a food was calculated as the average FII in 10 subjects.

$$\text{Food Insulin Index (FII)} = \frac{120\text{min AUC}_{\text{Insulin}} \text{ for 1000 kJ test food}}{120\text{min AUC}_{\text{Insulin}} \text{ for 1000 kJ reference food}} \times 100$$

The GS was calculated using a similar algorithm based on the blood glucose responses for the test and reference foods.

$$\text{Glucose Score (GS)} = \frac{120\text{min AUC}_{\text{Glucose}} \text{ for 1000 kJ test food}}{120\text{min AUC}_{\text{Glucose}} \text{ for 1000 kJ reference food}} \times 100$$

To determine the degree of association between different dietary factors and the postprandial physiological glycaemic and insulinaemic responses, linear regression analysis was used to test associations between FII and available carbohydrate, protein, fat, sugar, fibre, GS, GI and Glycaemic Load (GL) for all 147 single foods. A subanalysis was also used to test associations between FII and types of fat and select amino acids for foods for which more detailed nutritional information was available. Relationships between macronutrients and FII were also visualised as colour-coded response profiles using thin-plate spline procedures in R (version 3.0.2).

To validate the previously published FII stepwise linear regression equations¹⁸, the individual test subject's FII for the foods tested in this study were calculated using these equations and compared with the 254 actual observations. Correlations between the calculated FIIs and between the calculated and observed FII for the original 446 observations and the new 254 observations were analysed and compared. The combined 700 individual observations were used to generate a new stepwise regression equation to examine the extent to which the different predictors accounted for the variability of the observed postprandial responses.

All statistical analyses were carried out by using the SPSS statistical package version 21 (SPSS Inc., Chicago, IL, USA). Differences and correlation coefficients were considered statistically significant if the P value was < 0.05 and was highly significant if the P value was < 0.01 (2-tailed).

3.4 Results

There were large variations in the observed glucose and insulin responses to the 26 single foods, with GS ranging from 1 for cream to 100 for sweet potato, and FII ranging from 7 for brie cheese to 96 for sweet potato. There were large differences in FII both within and between food groups (**FIG**

3.1). Within this sample of foods, meat-based foods (including fresh cuts, deli meats and processed products such as chicken nuggets and meat pies) had an average FII of 25, dairy foods an average of 33, cereal and grain based products foods an average of 44, and vegetables (including legumes, starchy and non-starchy varieties) an average of 60.

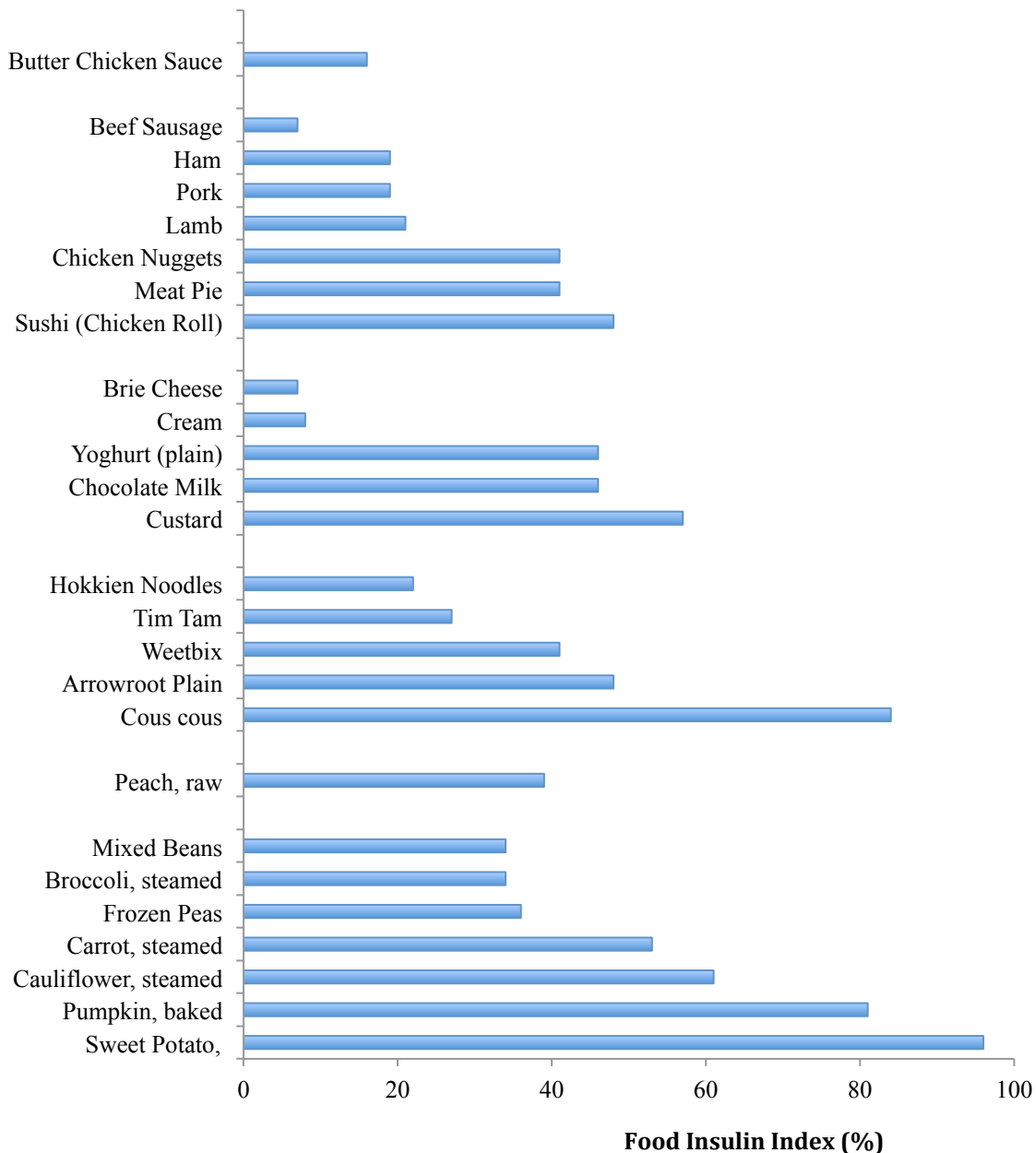


Figure 3.1: Observed insulin responses of 26 single foods, relative to an isoenergetic reference food (FII) arranged by food group.

Table 3.1: Macronutrient composition, glycaemic index (GI), glycaemic load (GL), actual glucose score (GS), and food insulin index (FII) for 1000-kJ portions of the reference glucose and test foods

Food	Test Date	Weight (g/MJ)	Protein (g/MJ)	Fat (g/MJ)	AvCHO (g/MJ)	Sugar (g/MJ)	Fibre (g/MJ)	GI (%)	GL (g/MJ)	GS (%)	FII (%)
Vegetables and legumes											
Peas, steamed from frozen (McCains) *	2011	333	17	1	21	7	22	22	5	40 ± 10	37 ± 8
Carrots, peeled and steamed (Australia) *	2011	775	9	2	32	29	33	33	14	35 ± 5	44 ± 7
Broccoli, steamed (Australia) *	2011	877	4	4	11	11	25	-	-	16 ± 5	29 ± 8
Cauliflower, steamed (Australia) *	2011	971	21	2	19	19	27	-	-	31 ± 7	48 ± 9
Sweet Potato, orange, peeled and steamed (Australia) *	2011	313	6	0	48	19	10	61	29	100 ± 11	96 ± 14
Butternut pumpkin, baked (Australia) *	2011	431	3	11	37	28	21	51	19	64 ± 15	77 ± 16
4 Bean Mix (Edgells)	2013	201	16	6	33	5	16	37	12	31	34
Fruits											
Peach, raw (Australia)	2011	565	7	1	51	46	13	56	29	53	39
Dairy products											
Chocolate milk (Moove)	2013	341	19	6	35	34	0	26	9	46	46
Yoghurt, plain (Dairy Farmers)	2013	213	10	7	34	33	0	18	6	22	46
Brie Cheese (Coles)	2013	65	11	21	1	1	0	-	-	3	7
Custard (Dairy Farmers)	2013	233	10	6	36	32	0	29	10	32	57
Cream (Dairy Farmers)	2013	72	2	25	2	2	0	-	-	1	8

Food	Test Date	Weight (g/MJ)	Protein (g/MJ)	Fat (g/MJ)	AvCHO (g/MJ)	Sugar (g/MJ)	Fibre (g/MJ)	GI (%)	GL (g/MJ)	GS (%)	FII (%)
Protein foods											
Lamb, grilled	2013	136	38	10	0	0	0	-	-	6	21
Pork, grilled	2013	239	53	3	0	0	0	-	-	3	19
Ham, shaved (Coles)	2013	213	34	11	0	0	0	-	-	10	19
Beef sausage, thin, grilled (Coles)	2013	106	14	19	35	0	0	-	-	7	7
Beef meat pie (Four'n'Twenty)	2013	104	10	12	23	0	-	-	-	35	41
Chicken nuggets (Ingham)	2013	99	18	13	18	1	-	46	8	29	41
Sushi, chicken roll (I Love Sushi)	2013	160	19	4	37	5	-	48	18	59	48
Carbohydrate foods											
Weetbix (Sanitarium)	2013	67	8	1	45	2	7	69	31	48	41
Plain Biscuit, Arrowroot (Arnotts)	2013	54	3	6	42	12	0	69	29	58	48
Tim Tam (Arnotts)	2013	46	2	12	30	20	0	-	-	32	27
Hokkien Noodles (Kan Tong)	2013	149	10	1	47	0	0	-	-	43	22
Couscous	2013	67	8	1	48	0	2	65	31	66	84
Butter chicken sauce (Sharwood's)	2013	209	3	16	21	9	0	-	-	22	16

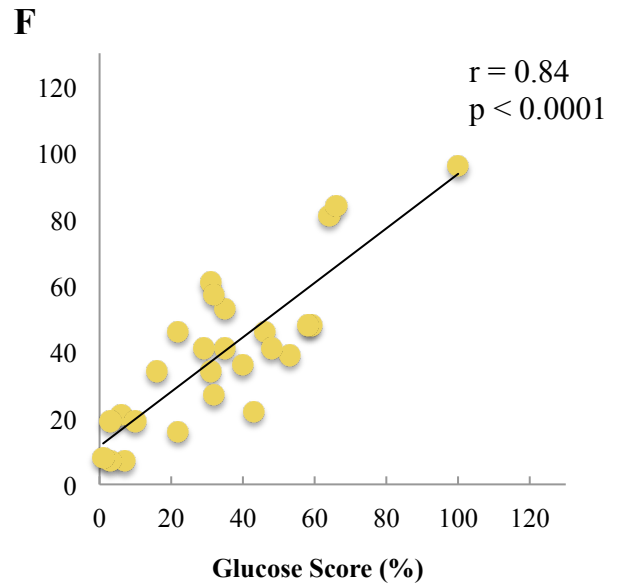
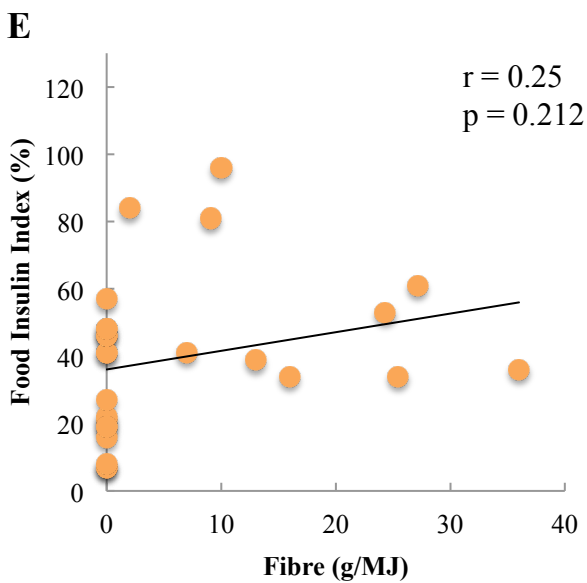
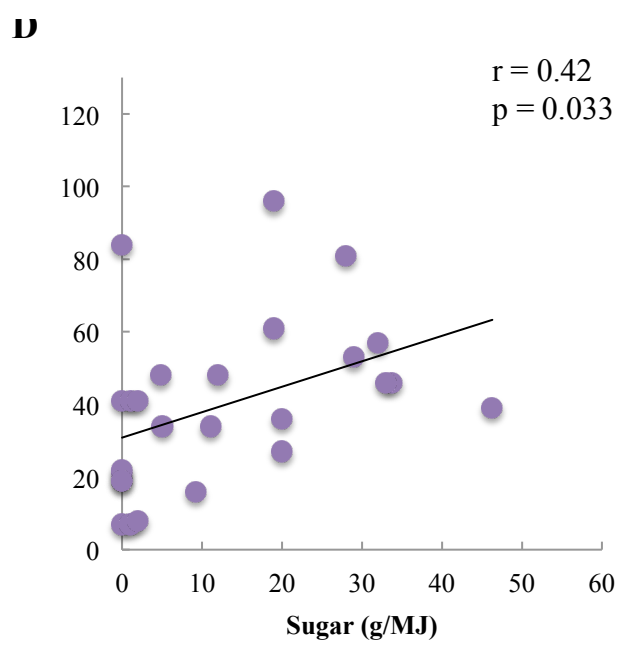
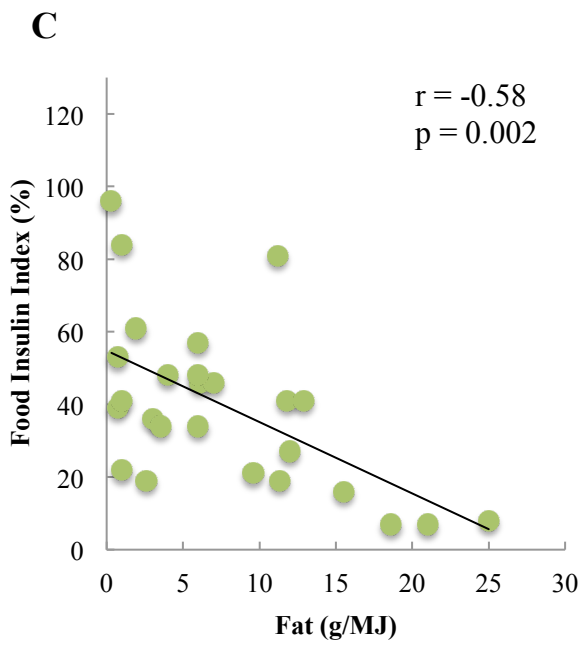
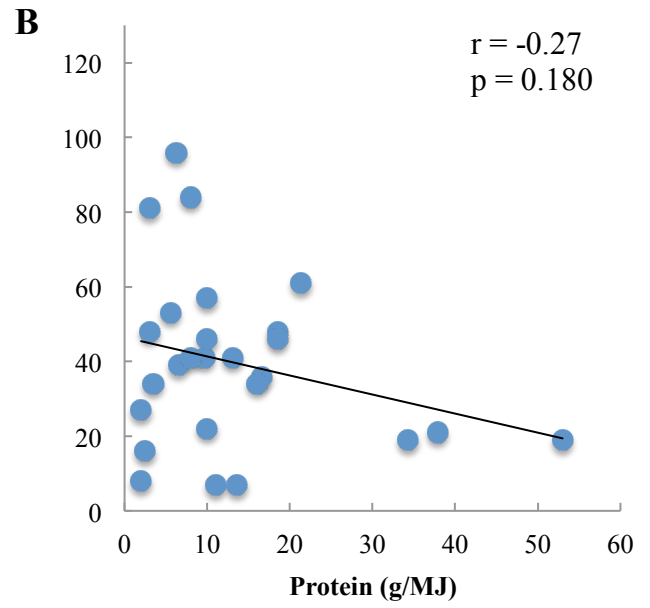
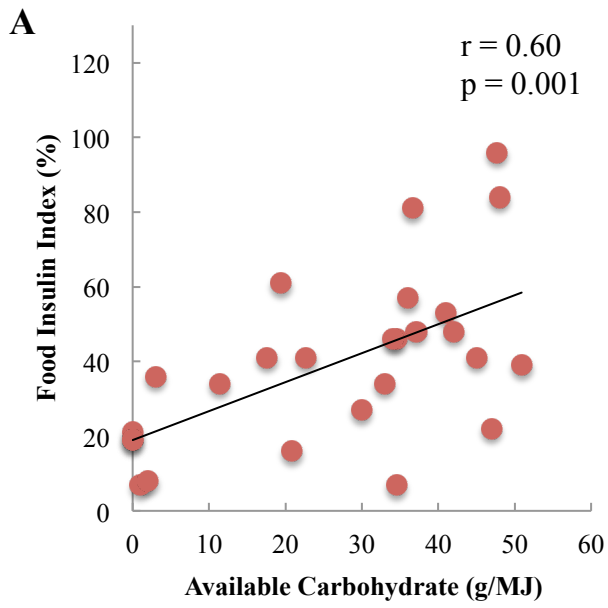
Mean ± SEM

* Tested in 300 kJ portion and GS and FII determined against 300 kJ Glucose.

Not surprisingly, the glucose responses were significantly correlated with the available carbohydrate, GI and GL of the tested foods ($r = 0.80$, $p < 0.0001$; 0.66 , $p = 0.010$ and 0.72 , $p = 0.004$ respectively). There were also significant inverse relationships between the GS and the protein and fat contents of the foods ($r = -0.40$, $p = 0.042$ and $r = -0.59$, $p = 0.001$ respectively). Sugar and fibre were not significantly associated with the GS.

Available carbohydrate, followed closely by fat, were the strongest individual predictors of the FII, explaining 36% and 34% of the variation in the observed insulin responses respectively. However, as with the glycaemic response, there was an inverse relationship between FII and the fat content of the food ($r = 0.60$, $p = 0.001$ for GS and $r = -0.58$, $p = 0.002$ for FII; **FIG 3.2 A-H**). Sugar was the only other significant predictor of the FII in these foods, explaining 18% of the variation.

Interestingly, protein was not a significant predictor of FII, despite the tested foods being dominated by those high in protein and/or fat and low in carbohydrate ($r = -0.27$, $p = 0.180$). In isolation, protein was a weak predictor of FII, explaining only 7% of the variation.



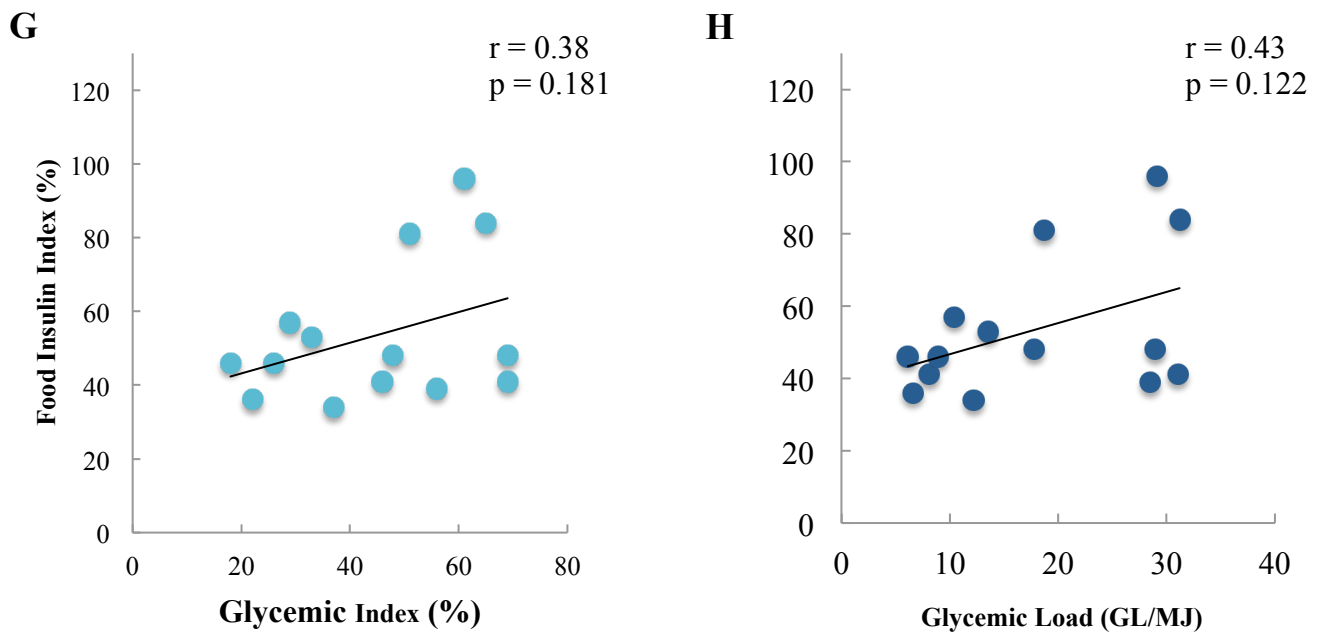


Figure 3.2 A-H: Univariate correlations between the food insulin index and the available carbohydrate, protein, fat, sugar, fibre, Glucose Score (GS), Glycaemic Index (GI) and Glycaemic Load (GL) for 1000 kJ portions of 26 single foods (G & H: $n = 14$).

Combining the 26 tested foods with the 121 previously published foods¹⁶ also demonstrated the wide range of FII values, with large variations within and between food groups (**FIG 3.3**).

The glucose responses to these 147 foods remained significantly correlated with the available carbohydrate, GI and GL of the foods, with the relationships strengthening with the inclusion of a greater range of foods ($r = 0.81, 0.85$ and 0.90 respectively, $p < 0.0001$ for all). GL was the strongest predictor of glycaemic variation, explaining 80% of the variation in the GS. GL was followed closely by GI, which explained 71%, and available carbohydrate, which explained 66% of the variation. The inverse relationships between GS and protein and fat were sustained, becoming highly significant with the expansion of the sample size ($r = -0.45$ and -0.61 respectively, $p < 0.001$ for both). Both sugar and fibre showed weak, positive associations with GS, however only sugar became a significant predictor of the FII ($r = 0.28$, $p = 0.001$ for sugar and $r = -0.12$, $p = 0.135$ for fibre).

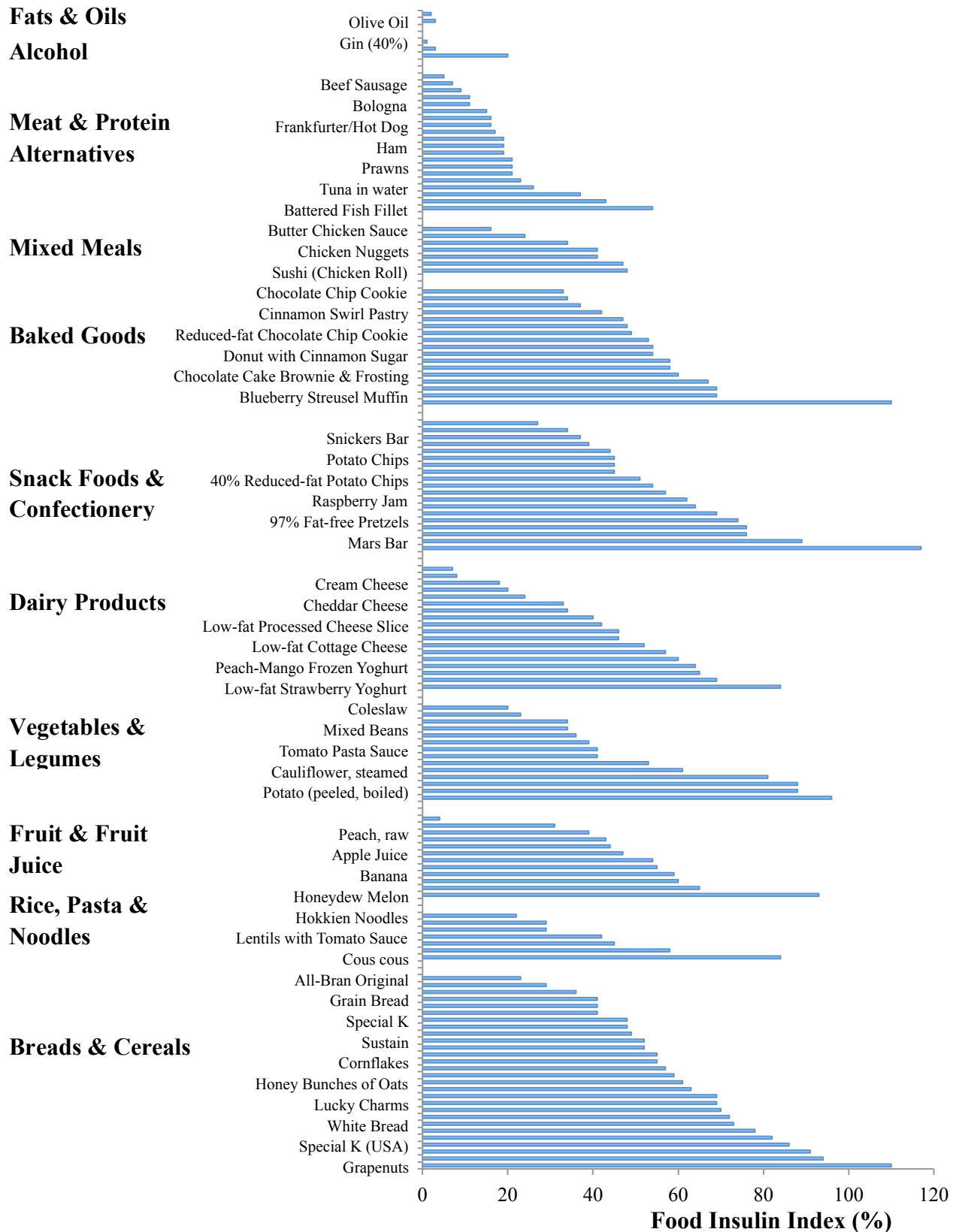
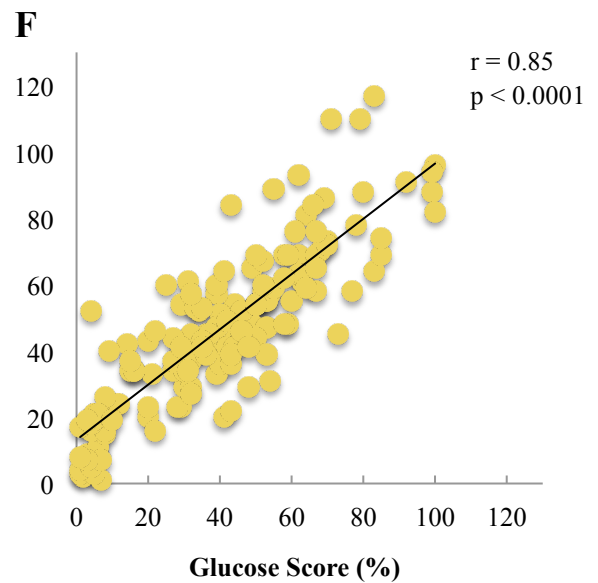
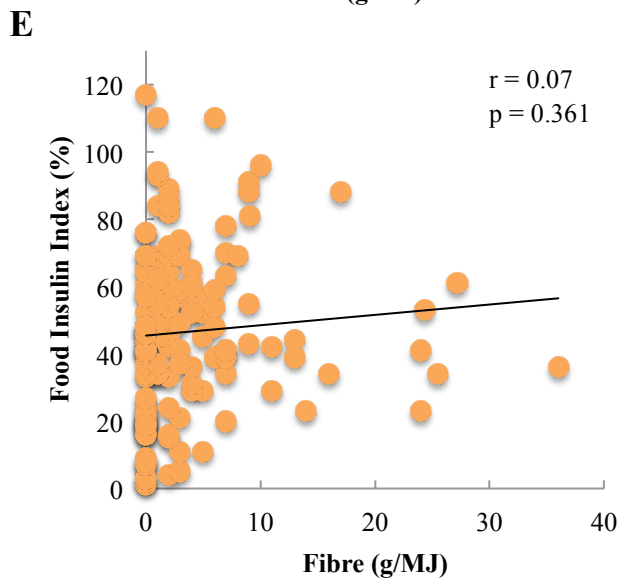
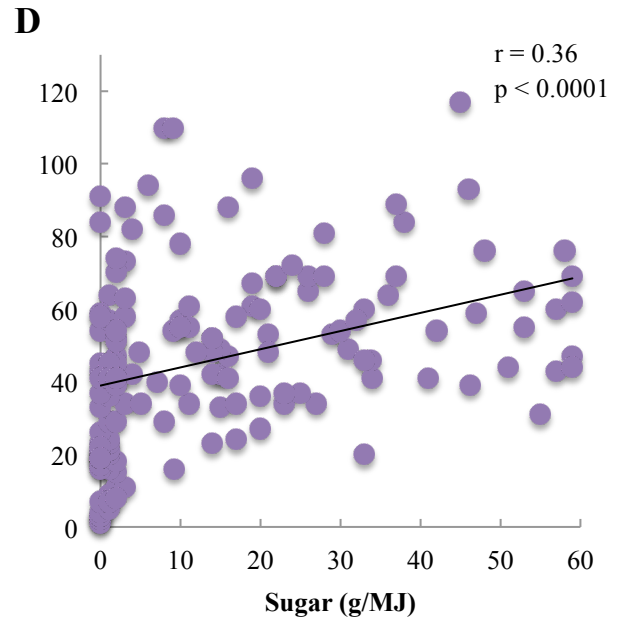
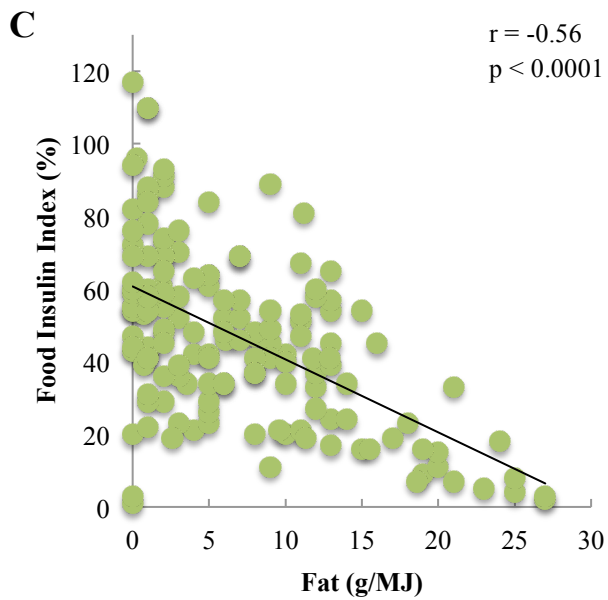
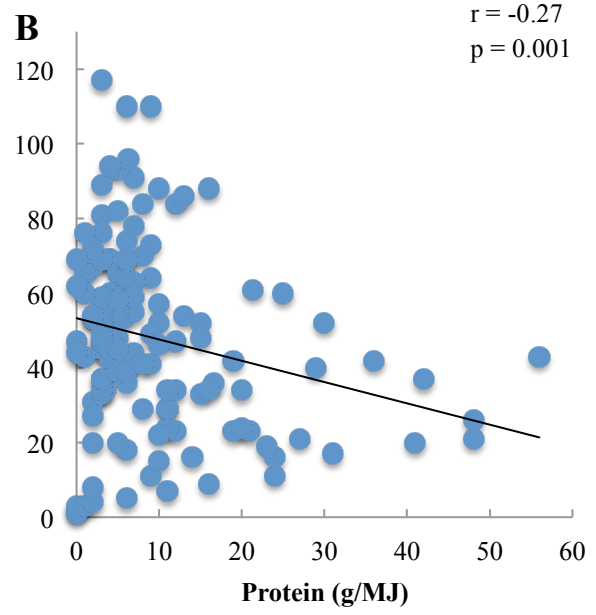
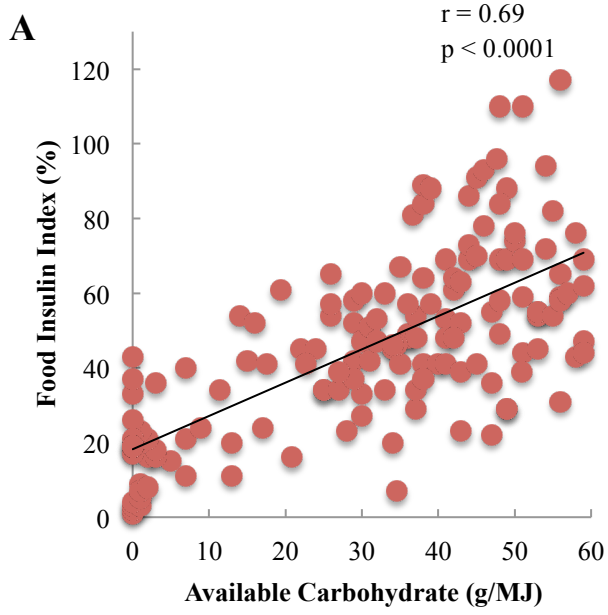


Figure 3.3: Observed insulin responses of 147 single foods, relative to an isoenergetic reference food (FII) arranged by food group.

GL, GI and available carbohydrate were also strongly and significantly correlated with the FII when the databases were combined ($r = 0.74, 0.72$ and 0.69 respectively, $p < 0.0001$ for all; **FIG 3.4: A, G & H**). Individually, GI explained 51% and available carbohydrate explained 47% of the variation in the observed insulin responses, while their mathematical product, GL, was the strongest individual predictor of the FII, explaining 55% of the variation in FII. Sugar was also moderately but significantly correlated with the FII ($r = 0.36, p < 0.0001$; **FIG 3.4D**).

With the inclusion of all tested foods, the association between the protein content of the food and the FII was relatively weak but highly significant ($r = -0.27, p = 0.01$; **FIG 3.4B**). The correlation between the FII and fat content of the foods remained moderately strong but now highly significant, with an inverse relationship ($r = -0.56, p < 0.0001$; **FIG 3.4C**). In isolation, fat explained 31% of the variation in FII and protein explained 7% of the variation.

Fibre was the only nutrient assessed that showed virtually no association with the FII ($r = 0.08, p = 0.361$ **FIG 3.4E**).



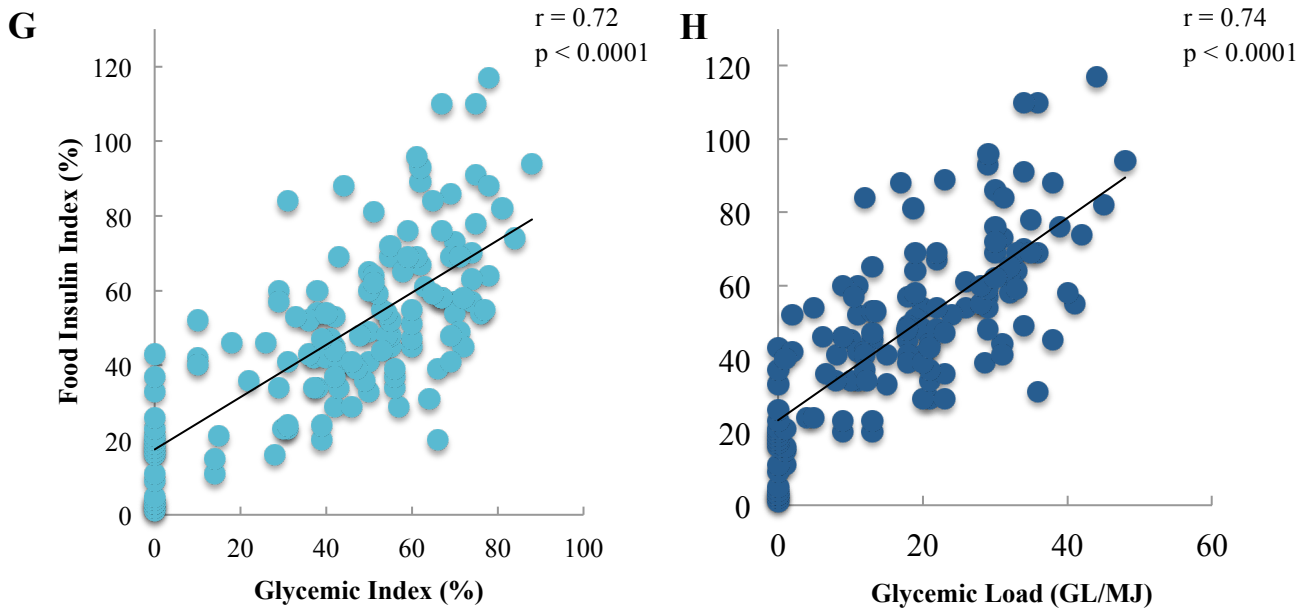


Figure 3.4 A-H: Univariate correlations between the food insulin index and the available carbohydrate, protein, fat, sugar, fibre, Glucose Score (GS), Glycaemic Index (GI) and Glycaemic Load (GL) for 1000 kJ portions of 148 single foods (G & H: $n = 135$).

Carbohydrate, protein and fat contents of each 1000 kJ food portion were mapped against FII as colour-coded response profiles to compare the macronutrients and establish a hierarchy based on their insulinotropic ability (**FIG. 3.5: A-C**). The response profiles visualise the trends in FII as three dimensional maps, with the 3 macronutrients mapped on 3 different axes (X, Y and Z) and the FII values represented by colour gradations. The red regions indicate the highest FII and the dark blue regions indicate the lowest FII. The three-dimensional plots are presented in two-dimensions by depicting the cross-sectional view of the plot at the median value of the third variable (median value is presented below the a-axis in parentheses). For example, in **FIG. 3.5A**, protein is shown on the x-axis, carbohydrate is shown on the y-axis and fat is on the z-axis (but cannot be shown in 2D). The displayed figure is the cross-section of the graph at the median fat content value (i.e. 5 g

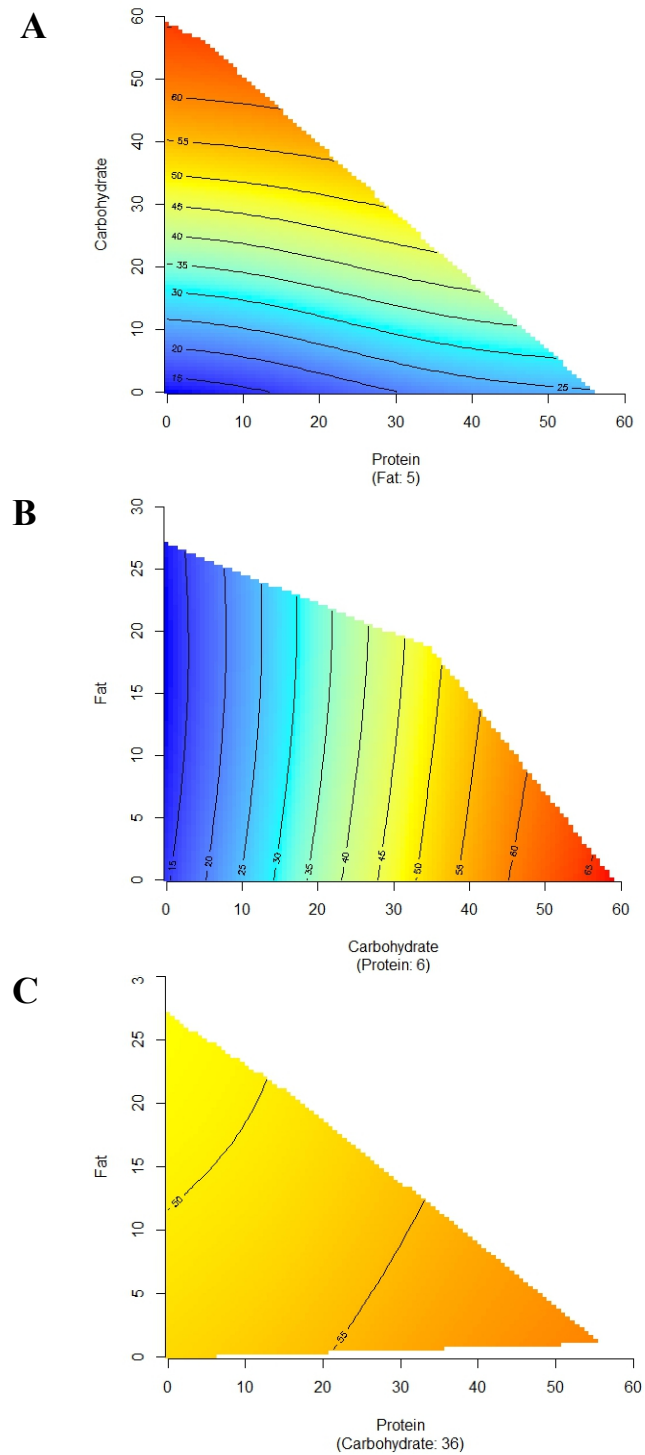


Figure 3.5 A–C: Colour-coded response profiles showing the relationship between Food Insulin Index versus carbohydrate, protein and fat. Three 2D slices are presented to show all three nutrient dimensions. For each 2D slice, the third factor is shown at the median (presented below the x-axis in parentheses). In all surfaces, the red region indicates the highest FII and the dark blue region indicates the lowest FII.

of fat/1000 kJ). **FIG. 3.5B** is cut at the median protein value: 6 g of protein/1000 kJ and **FIG. 3.5C** is cut at the median carbohydrate value: 36 g of carbohydrate/1000 kJ. The plots show that carbohydrate is the dominant macronutrient determining postprandial insulin responses, producing the largest increases in FII with increasing carbohydrate content compared with both protein and fat (**FIG. 3.5: A & B**). Plotting fat against protein shows that in the hierarchy of macronutrients, protein is slightly more insulinotropic than fat, with a trend towards a slightly higher FII with increasing protein content (**FIG. 3.5C**).

To further elucidate whether the type of fat influenced insulin secretion, saturated, polyunsaturated, and monounsaturated fat content per 1000 kJ were correlated with the FII. All 3 types of fat had moderately strong, highly significant inverse associations ($r = -0.42, -0.37$ and -0.51 respectively, $p < 0.0001$ for all; **FIG 3.6A-C**). Monounsaturated fat was the strongest individual predictor of the FII, explaining 26% of the variation, followed by saturated fat explaining 17% and then polyunsaturated fat explaining 16% of the variation.

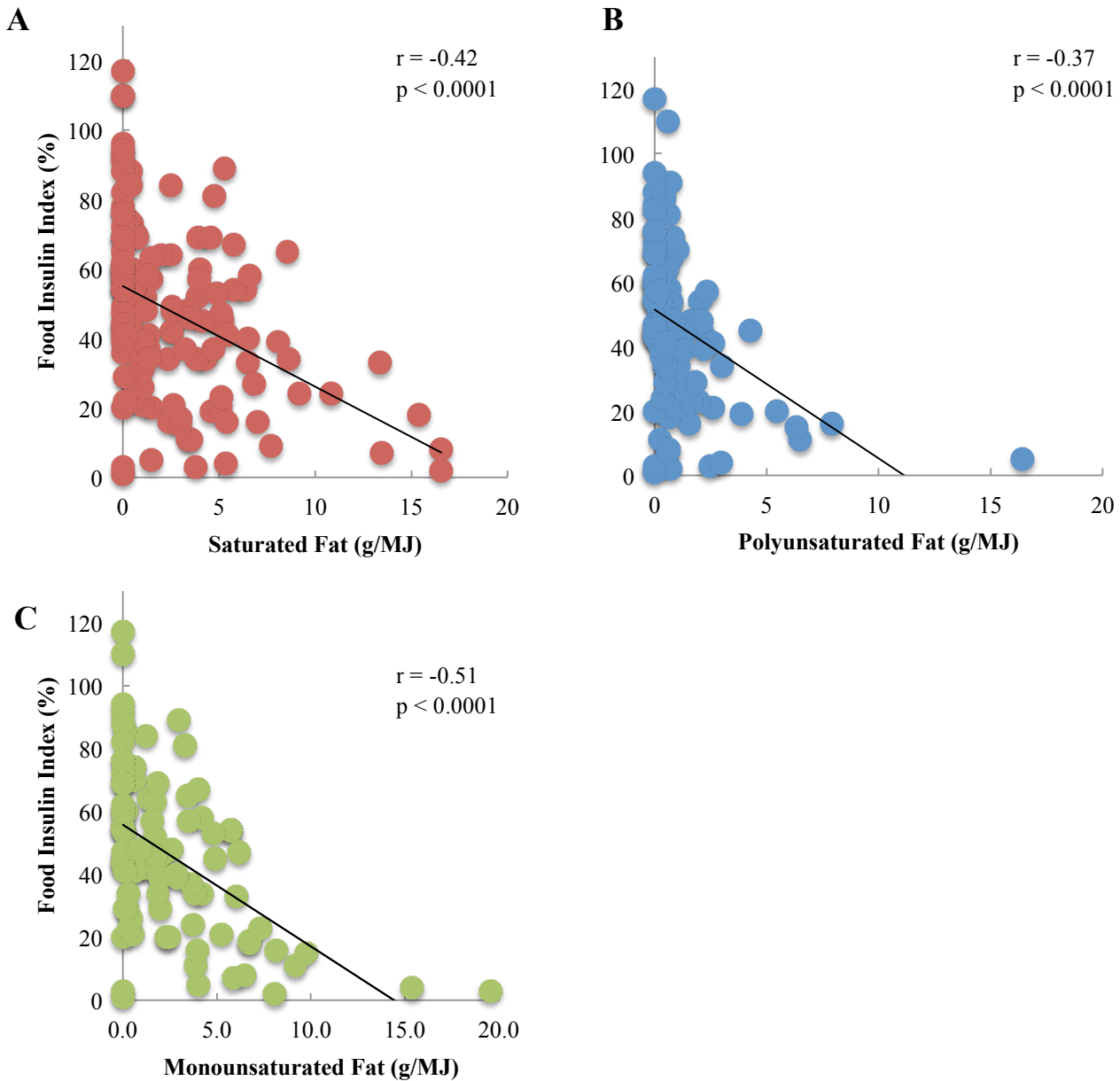


Figure 3.6 A-C: Univariate correlations between the food insulin index and the saturated fat, polyunsaturated fat and monounsaturated fat for 1000 kJ portions. A: $n = 147$ single foods, B & C: $n = 99$ single foods.

As carbohydrate displaces fat and protein within foods, foods containing <10 g of carbohydrate/1000 kJ were analysed separately (**FIG 3.7: A-D**). Amongst these 29 low-carbohydrate foods, the relationship between total fat and FII was weakened but remained inverse and significant ($r = -0.40$, $p = 0.030$). However, none of the individual types of fat assessed was significant or strongly

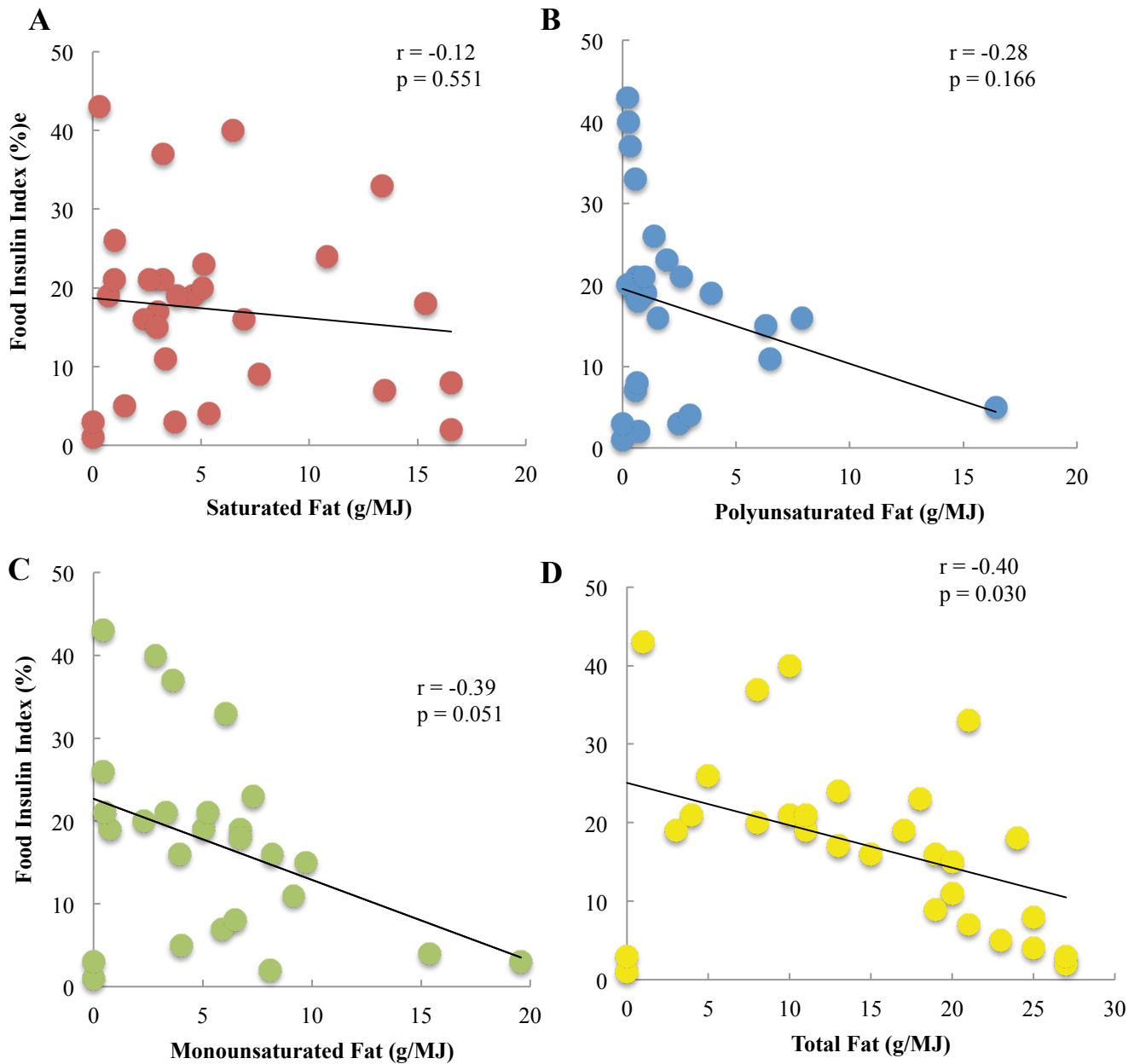


Figure 3.7 A-D: Univariate correlations between the food insulin index and the total fat, saturated fat, polyunsaturated fat and monounsaturated fat for 1000 kJ portions of foods containing 10 g of available carbohydrate or less. A & B: $n = 29$ single foods, C & D: $n = 27$ single foods.

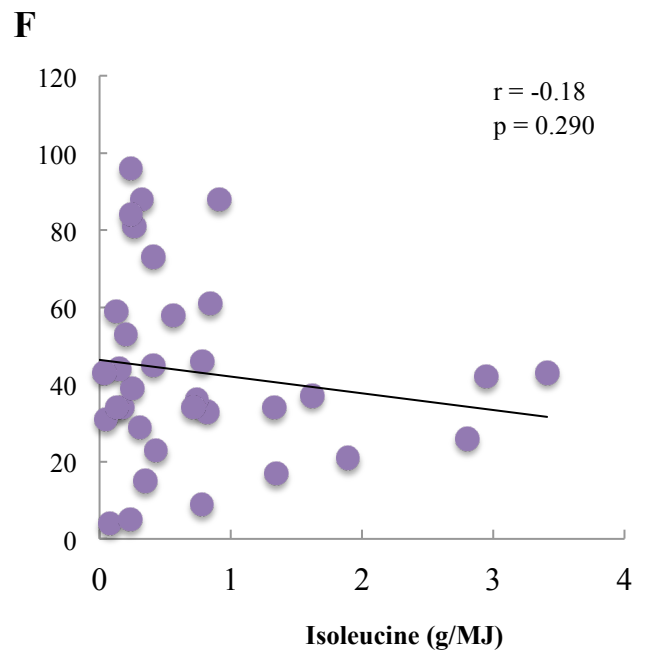
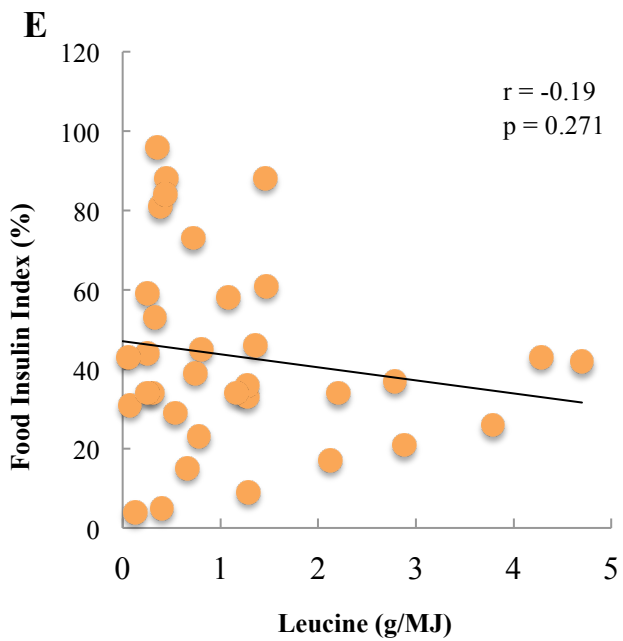
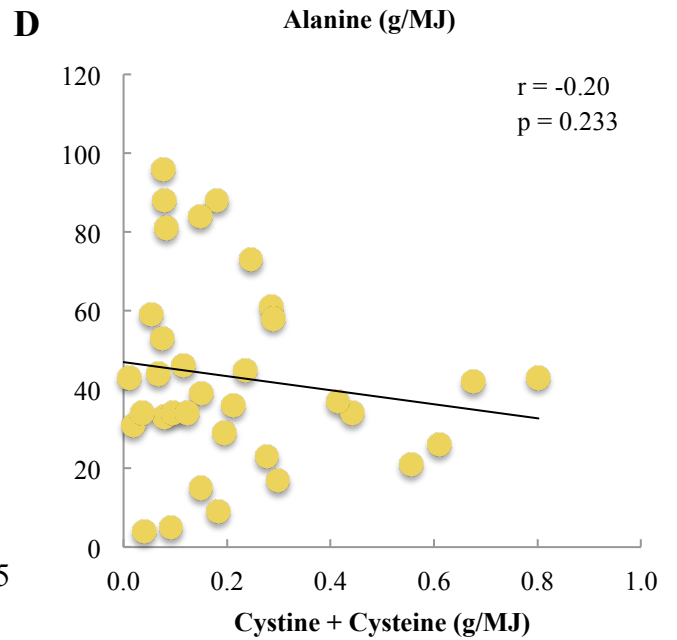
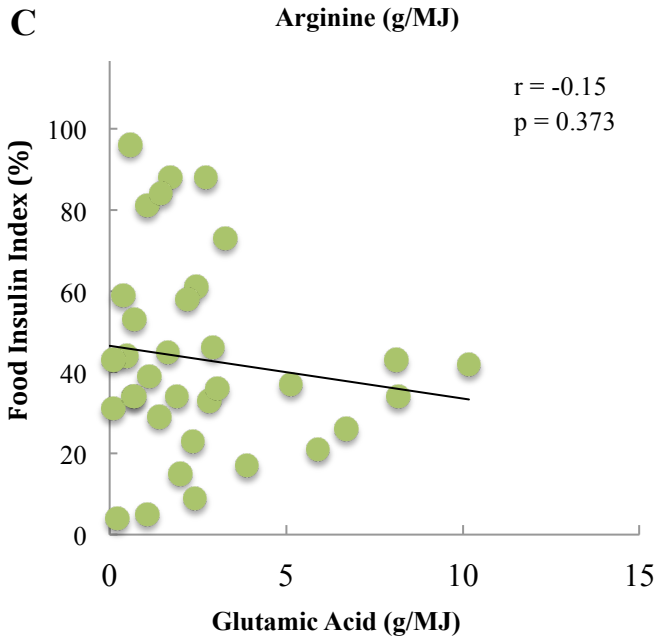
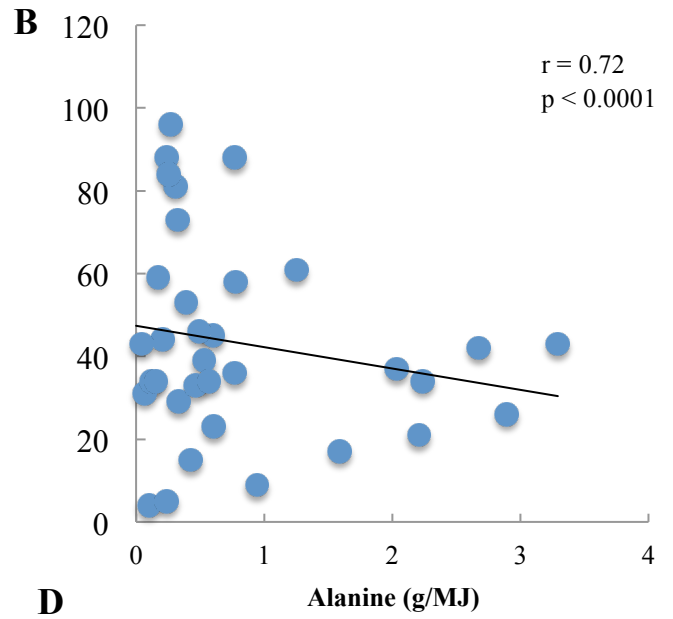
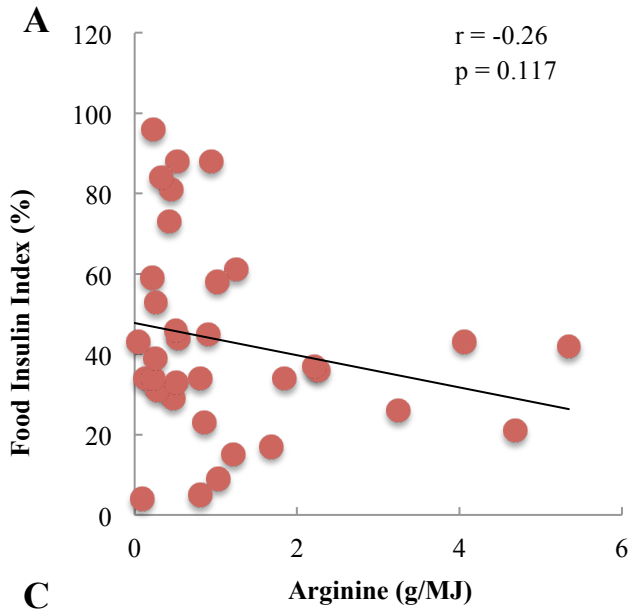
correlated ($r = -0.12$, $p = 0.551$ for saturated fat, $r = -0.28$, $p = 0.166$ for polyunsaturated fat and $r = -0.39$, $p = 0.051$ for monounsaturated fat). Monounsaturated fat remained the strongest predictor of the three types of fat but was weakened noticeably, accounting for only 15% of the variation in the

FII in low carbohydrate foods. Saturated and polyunsaturated fats also weakened dramatically, now accounting for only 1% and 8% of the variation respectively.

To further investigate the effect of differing types of proteins, 7 key insulinotropic amino acids were correlated with the FII. Amongst the 147 foods in the FII database, the amino acid content could be located for 37 foods. The amino acids were all only weakly correlated with the observed insulin responses and none was significant (**FIG 3.8:A-G**).

However, when only *low* carbohydrate foods were included in the analysis, total protein was strongly and positively associated with the FII and highly significant ($r = 0.73$, $p < 0.0001$).

Individually, protein accounted for more than half the variation in FII in low carbohydrate foods ($r^2 = 54\%$). Furthermore, the relationships between the amino acids and the FII became positive and 6 of the 7 identified amino acids were strongly and significantly correlated with the FII (**FIG 3.9: A-H**). Of the amino acids, the BCAA were among the strongest predictors of the FII, with isoleucine accounting for 52%, leucine accounting for 49% and valine accounting for 44% of the variation in insulinaemia ($p = 0.008$, 0.017 and 0.020 respectively). Arginine and alanine were also significant predictors, accounting for 49% and 44% of the variation respectively ($p = 0.018$ and 0.020 respectively). Cystine + Cysteine were the weakest predictors, accounting for only 24% of the variation and not significant ($p = 0.109$).



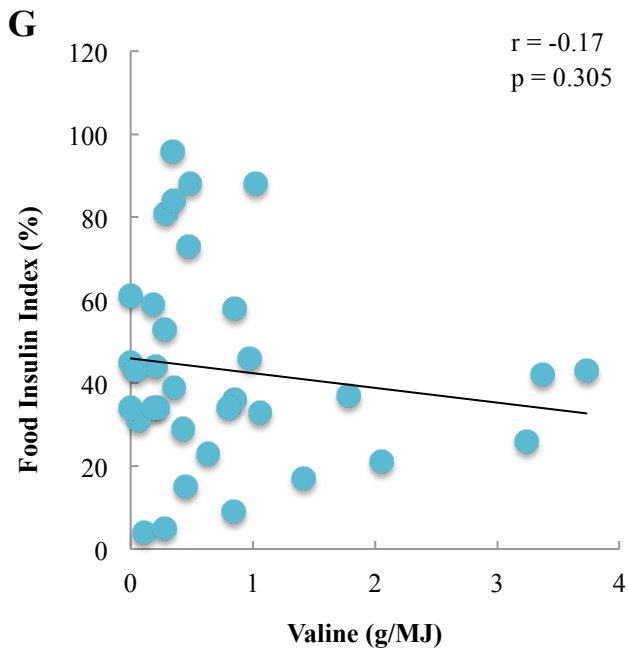
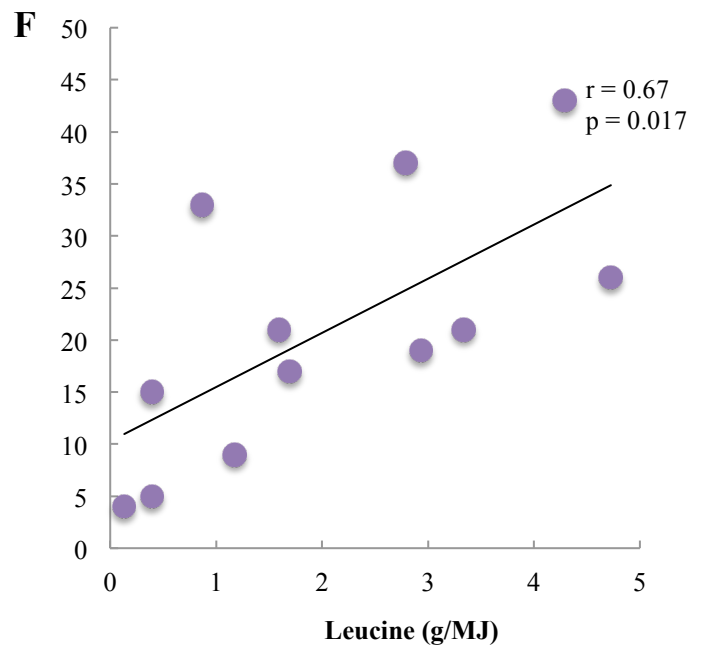
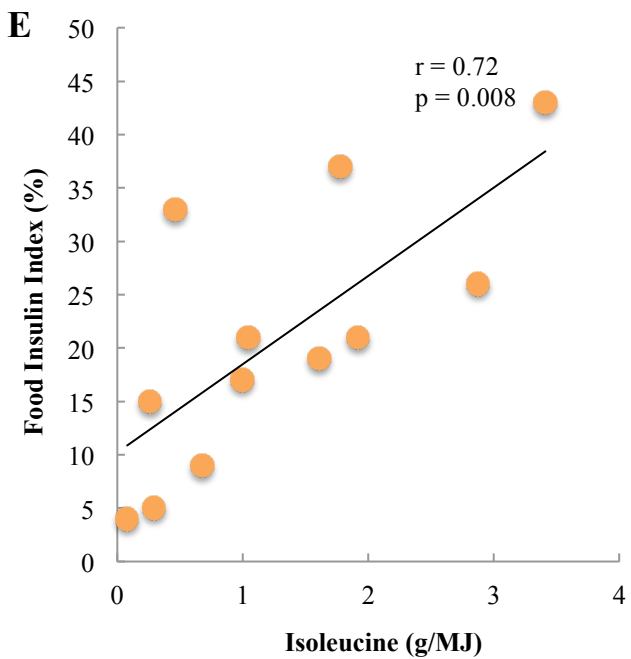
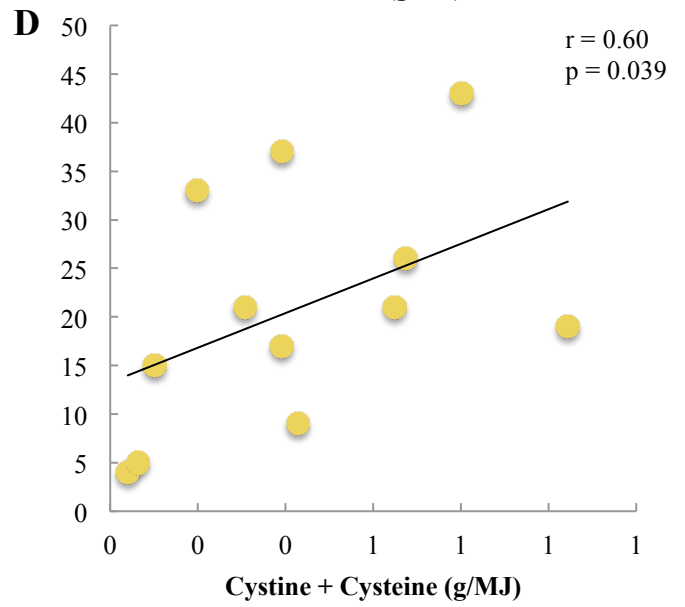
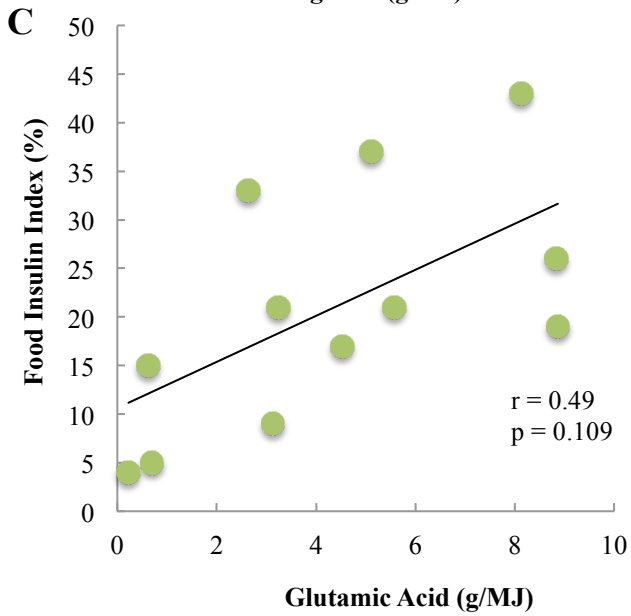
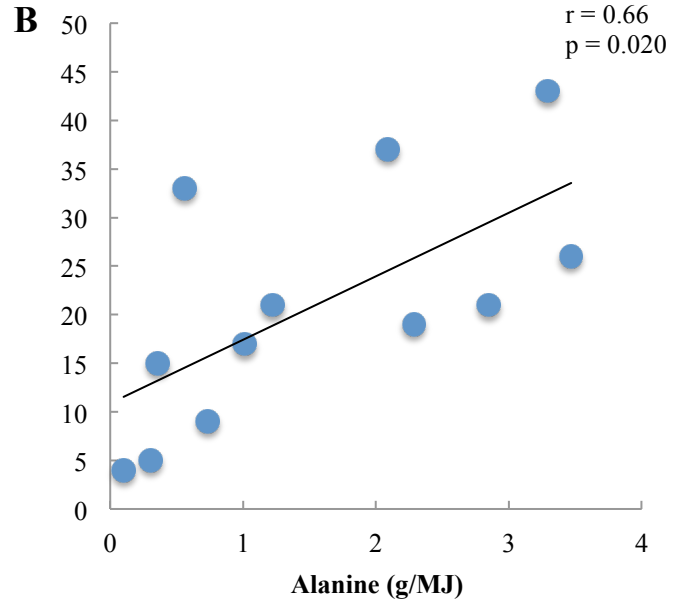
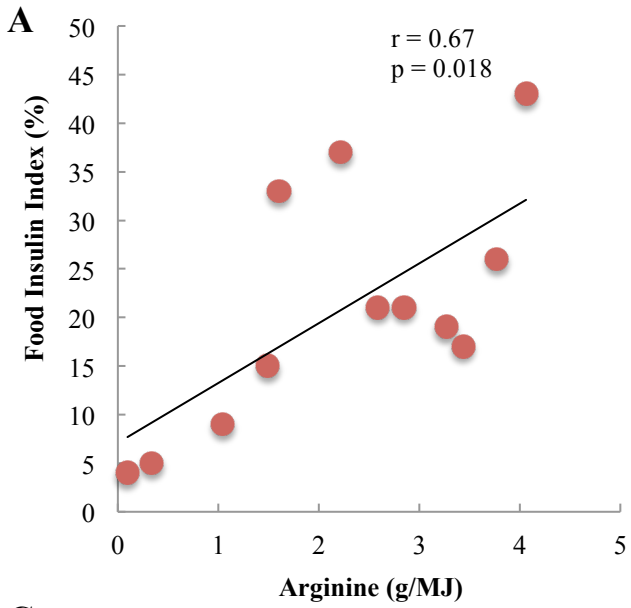


Figure 3.8 A-G: Univariate correlations between the food insulin index and alanine, glutamic acid, arginine, cystine + cysteine, leucine, isoleucine and valine for 1000 kJ portions of 37 single foods.



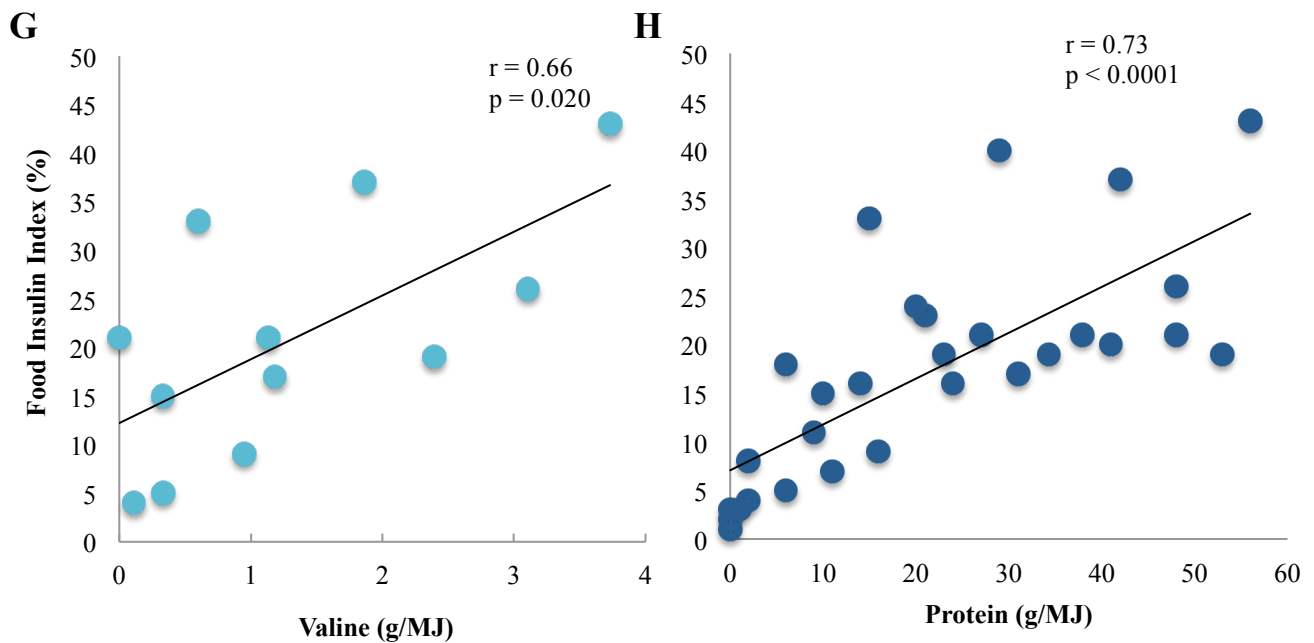


Figure 3.9 A-G: Univariate correlations between the food insulin index and alanine, glutamic acid, arginine, cystine + cysteine, leucine, isoleucine and valine for 1000 kJ portions of single foods containing at least 10g of available carbohydrate (A-G: $n = 12$, H: $n = 29$).

The previously published prediction equations for the FII can be validated by using the regression equations to calculate the FII for individual observations and comparing with the 254 individual FII observations for the 26 foods in the present study. The correlations were 0.38 and 0.31 respectively for the 2 equations and, although both equations were highly significant ($p < 0.0001$), they were able to account for only 14% and 10 % of the variation in the FII respectively. The correlation coefficient between the two equations was 0.95 ($p < 0.0001$).

Given the calculated FII equations were not strong predictors of the observed FII, a new stepwise multiple linear regression analysis of the average FII for the 147 foods was performed (**Equation 1**).

$$\text{FII} = -4.2 + 0.9 \text{ GS} + 0.3 \text{ Sugar} + 0.5 \text{ Protein} + 0.4 \text{ Fat} \text{ (Equation 1)}$$

GS, sugar and protein were selected as highly significant predictors ($p < 0.001$) whereas fat did not reach significance ($p = 0.053$) and available carbohydrate, GI and GL were not selected. Together, GS, sugar and protein accounted for 78% of the variation in the observed FII.

Removing fat gives the following equation (**Equation 2**):

$$\text{FII} = 4.0 + 0.9 \text{ GS} + 0.2 \text{ Sugar} + 0.4 \text{ Protein} \text{ (2)}$$

All variables were highly significant ($P < 0.001$) and the equation accounts for 77% of the variation in FII.

Equations 1 and 2 both selected GS as the most important predictor of FII. However, to obtain a food's GS, the same methodology for FII testing needs to be undertaken and thus the FII could also be measured in tandem, making calculating FII using these equations superfluous. Alternatively, the GL could be used in place of GS (correlation coefficient between GL and GS, $r = 0.90$). GL can be calculated from the known carbohydrate content and published GI (**Equation 3**).

$$\text{FII} = 16.3 + 0.4 \text{ Protein} + 1.5 \text{ GL} \text{ (Equation 3)}$$

In this scenario, both protein and GL were significant predictors of FII (protein: $p = 0.013$; GL: $p < 0.0001$) and the equation accounts for 57% variation.

Since GL still requires in vivo testing in order to determine the prerequisite GI, a fourth regression equation was developed using only the nutritional composition of the food. Including all nutrients in the database, the equation explains only half of the variation in the FII ($r^2 = 50\%$) with carbohydrate as the only significant variable ($p < 0.0001$) (**Equation 4**).

$$\text{FII} = 16.2 + 1.0 \text{ Carbohydrate} - 0.2 \text{ Fat} + 0.3 \text{ Protein} - 0.1 \text{ Sugar} - 0.4 \text{ Fibre} \text{ (Equation 4)}$$

When only significant predictors of FII were included in the equation, the model selected carbohydrate ($p < 0.001$) and protein ($p = 0.032$) (**Equation 5**). The overall equation explained slightly less of the variation than Equation 4 (49% vs 50%).

$$\text{FII} = 10.4 + 1.0 \text{ Carbohydrate} + 0.4 \text{ Protein} \text{ (Equation 5)}$$

3.5 Discussion

The results of this study add to the growing body of knowledge of the relationship between whole foods, specific dietary factors and insulinaemia. It is clear that a simple food group approach is not sufficient because there were large differences in the GS and FII both within and between food groups. Foods with similar macronutrient contents, such as hokkien noodles and sweet potato had a more than 4-fold difference in FII despite containing almost identical amounts of carbohydrate, protein and fat per MJ (FII = 22 vs. 96). Among the 26 tested foods for this thesis, carbohydrate and fat were significant predictors of the FII explaining 36% and 34% of the variation in insulinaemia individually. However, when these foods were combined with the 121 previously

tested foods, GL, GI and available carbohydrate became the strongest predictors of the FII, explaining 55%, 51% and 47% of variation respectively. This was a weaker relationship than had previously been reported¹⁶ (84%, 76% and 66% respectively), but not surprising in light of the number of meat-based products and dairy produce represented in the present study, which when added to the database, reduced the proportion of starchy, carbohydrate-rich foods.

Fat, protein and sugar were all also significant but relatively weak predictors, accounting for only 31%, 8% and 13% of the variation respectively. Together the average GS, sugar, protein and fat could be used to predict the final average FII value, explaining ~80% of the variation in insulinaemia. However, alone without the GS, macronutrients explained only 50% of the variation. These findings indicate that a precise FII cannot be generated on the macronutrient composition alone and therefore in vivo testing is required to obtain the GS or the FII. Clearly, normal physiological insulin secretion is a multifaceted process involving complex nutritional and metabolic interactions between nutrients and the food matrix before and after digestion and absorption.

Despite this intrinsic complexity, the results of these analyses suggest that there is a hierarchy of macronutrients in relation to their potency as insulin secretagogues. Given the strength of the correlations and the colour-coded response profiles, available carbohydrate is easily identifiable as the predominant macronutrient increasing insulin secretion. This is in agreement with the scientific literature and clinical practice, which universally accepts that carbohydrate is the predominant macronutrient responsible for raising blood glucose levels, and therefore the most potent stimulus for insulin secretion. Yet carbohydrate is clearly not the sole macronutrient involved because by itself, it could account for only 47% of the variation in insulinemia, and foods containing the same amount of available carbohydrate (eg 50 g) showed more than 4-fold range in FII (**FIG 3.4A**). Indeed, carbohydrate was not selected as a predictor of FII in the multiple regression analysis

(Equations 1-3). It was only when the model was forced to drop GS, GL and GI, that carbohydrate was selected **(Equations 4-5).**

It is also interesting to note that the foods with the highest FII are commonly regarded as healthy foods: potatoes, pumpkin, baked beans, yoghurt, melons, couscous, cereals and breads. Sweet potato also posed an interesting paradox as it has a low-to-intermediate GI value and is encouraged as an alternative to high GI white potatoes for improving glycaemic control²⁰. Yet our results show that the sweet potato had a GS of 100 and a FII of 96, one of the most glycaemic and insulinaemic foods tested. When the FII is predicted using Equation 1 (ie using GS) the FII is calculated as 95, however when Equation 4 is used (ie using GL), the FII is considerably lower (62). This suggests that the GL and GS are not comparable between these tests, potentially due to the variety of sweet potato and cooking method used.

In the context of the complete FII database, protein appears to have an inverse relationship with the observed insulin responses and is a relatively weak predictor of the FII **(FIG 3.2B)**. This is in direct contrast to the literature, which identifies protein as a potent secretagogue. Reanalysis of the association between FII and protein involving only the low carbohydrate foods, reveals total protein had a highly significant, strong, positive correlation with the FII. This may help explain how high protein foods across different food groups tested in this study such as pork, lamb, yoghurt and milk all produced notable FII values, in some cases equivalent to their high carbohydrate, low protein counterparts. For example, grilled lamb fillets (a protein food with no carbohydrate) and hokkien noodles (a carbohydrate food) have an almost identical FII of 22 and 21. In reality, however, a higher protein diet will often be a lower carbohydrate diet. Thus protein tends to displace carbohydrate, the most potent insulin secretagogue.

While the relationship between protein and FII becomes positive when only low-carbohydrate foods were considered, the same was not true for fat and FII. Fat had an inverse relationship with FII

when low-carbohydrate foods as well as the entire database were considered. This indicates that a higher fat content lowers the FII under both circumstances. This may be because fat slows gastric emptying and therefore the digestion and absorption of carbohydrate, the primary insulin secretagogue. Fatty acids themselves do not directly stimulate insulin secretion and, in isolation, are relatively weak secretagogues. However, when *added* to a glucose load, they are able to significantly amplify glucose-stimulated insulin secretion beyond that of glucose alone²¹. The insulinotropic effect of fat may also be modulated by the type of fat within the food. The literature suggests that the potency of fat on insulin secretion is correlated with the degree of unsaturation, although the findings are not consistent. In vivo studies indicate that the postprandial insulin response is essentially unaffected by the type of fat^{22, 23}. This is consistent with the present findings where we found there were moderately strong, inverse relationships between fat and FII, irrespective of the type of fat. This may have been because we tested iso-energetic portions of food and carbohydrate, the strongest insulinotropic macronutrient, displaced fat and vice versa. When only low carbohydrate foods were considered (ie foods high in protein or fat or both), the relationship between fat and FII remained inverse, most likely because protein is more insulinotropic than fat, and fat slows the digestion of protein.

This chapter also attempted to elucidate the effect of different types of protein by examining the extent of the associations between the FII and specific insulinotropic amino acids. Of the amino acids assessed, the BCAA, leucine, isoleucine and valine, were the strongest individual predictors of the FII for low carbohydrate foods. This finding parallels the literature, which commonly reports these amino acids are potent secretagogues^{9, 10}, attributing it to their efficacy to their rapid digestion and activation of mTOR, which increases insulin secretion²⁴. The efficacy of dairy products in stimulating insulin secretion despite their low GI, has been attributed to their high BCAA content. During the cheese manufacturing process, milk is separated into curds and whey, and whey in particular is high in BCAA. This may explain why the milk-based products in our study such as

custard (FII = 57), plain yoghurt (FII = 46) and chocolate milk (FII = 46), as well as other reported in the literature such as low-fat strawberry yoghurt (FII = 84)¹⁶ and vanilla ice-cream (FII = 65)¹⁶ had a much higher FII than the cheeses, including brie cheese (FII = 7), cheddar cheese (FII = 33)¹⁶ and cream cheese (FII = 18)¹⁶, which have had the whey component, and thus the BCAA, removed.

We also sought to validate the accuracy of the previously published¹⁸ FII prediction equations, a practice that is highly recommended yet rarely done in the literature. The original regression analysis of the 446 individual FII observations for 38 different single foods yielded two equations, one including fat but not protein and the other including protein but not fat. The correlations achieved between the calculated FIIs using these equations and the actual observed FII for the foods in this study were lower than that published in the original paper (0.38 and 0.31 vs. 0.49 and 0.48), although these lower correlation coefficients are to be expected given the original equations were developed from the original set of data and foods. Given the calculated FII equations were not strong predictors of the observed FII, a new stepwise multiple linear regression analysis using the average FII for the 147 foods was performed. The new equations explained 77% of the variation in insulinaemia compared with just 32% for both of the original equations.

The FII and GS testing is a relatively new procedure, although the protocol parallels the International Standard of Operation (ISO) for GI testing to ensure reliable data. Because glucose and insulin responses are both highly variable within and between subjects, subjects acted as their own control, ie their glycaemic and insulinaemic responses to each test food was compared with their response to the reference food, thus minimising interindividual differences. Repeat tests of the reference food (tested 3 times) was also applied to reduce intra-individual variation and ensure greater precision. Furthermore, glucose and insulin sampling was done using capillary blood rather than venous blood as incremental glycaemic responses display a greater magnitude and less variability when measured in capillary blood²⁵.

The FII is determined by 120 min test sessions as per the GI testing ISO. In healthy subjects, the physiologic insulin release will have stabilised postprandial glycaemia and returned it to the baseline level by 120 min and therefore longer test sessions are unnecessary²⁶. Potentially, the same is not true for insulin secretion, particularly for foods high in protein and fat, and further studies with longer test sessions (3-5 h) are warranted to compare the iAUC.

Although a further 26 single foods were added to the FII database as a result of this study, additional testing of branded foods needs to be undertaken in order to develop a more comprehensive database of foods. This will be necessary if the FII is to be widely incorporated into clinical practice. This testing will also allow further exploration of the relationship between dietary factors and physiological insulin secretion evoked by foods and dietary factors.

In conclusion, the present study found wide variations in the observed insulin responses both within and between food groups. The FII could not be accurately calculated based on carbohydrate content alone or the full nutritional composition of the food, implying that in vivo testing is required. Correlations between the FII and different nutrients indicate that the postprandial insulin response is not the effect of a single nutrient but rather the nutritional and metabolic *interactions* between nutrients and the food matrix.

3.6 References

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CHAPTER 4

Estimating insulin demand for
protein-containing foods using
the Food Insulin Index

4.1 Abstract

Objective: The Food Insulin Index (FII) is a novel algorithm for ranking foods based on insulin responses in healthy subjects relative to an isoenergetic reference food. Our aim was to compare postprandial glycaemic responses in adults with type 1 diabetes who used both carbohydrate counting and the FII algorithm to estimate the insulin dosage for a variety of protein-containing foods.

Methods: 11 adults on insulin pump therapy consumed 6 individual foods (steak, battered fish, poached eggs, low fat yoghurt, baked beans and peanuts) on two occasions in random order, with the insulin dose determined once by the FII algorithm, and once with carbohydrate counting. Postprandial glycaemia was measured in capillary blood glucose samples at 30 min intervals over 3 h. Researchers and participants were blinded to treatment.

Results: Compared with carbohydrate counting, the FII algorithm significantly reduced mean blood glucose level (5.7 ± 0.2 mmol/L vs 6.5 ± 0.2 mmol/L, $p = 0.003$) and mean change in blood glucose level (-0.7 ± 0.2 mmol/L vs 0.1 ± 0.2 mmol/L, $p = 0.001$). Peak blood glucose was reached earlier using the FII algorithm than using carbohydrate counting (34 ± 5 vs 56 ± 7 min, $p = 0.007$). The risk of hypoglycaemia was similar in both treatments (48% vs 33% for FII vs carbohydrate counting respectively, $p = 0.155$).

Conclusions: In adults with type 1 diabetes, compared with carbohydrate counting, the novel FII algorithm improved postprandial hyperglycaemia after consumption of protein-containing foods.

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4.2 Introduction

Type 1 diabetes is an autoimmune disorder resulting in an absolute endogenous insulin deficiency that is characterised by chronic hyperglycaemia. Effective management of type 1 diabetes requires exogenous insulin to be closely matched to physiological demand in order to maintain optimal blood glucose control.

Carbohydrate counting is considered the foundation of intensive insulin therapy, whereby bolus insulin doses are matched to the carbohydrate content of the meal¹. Despite significant advancements in insulin technology, optimum postprandial glycaemic control remains difficult to achieve. Even patients within target glycated haemoglobin A1c (HbA1c) levels continue to experience unanticipated hyper- and hypoglycaemia, particularly in response to meals high in protein and/or fat²⁻⁶. Given the risk of developing life-threatening acute and chronic diabetic complications, improving the insulin dose algorithm presents a significant clinical issue.

The Food Insulin Index (FII) is a novel algorithm of ranking foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food⁷. Using food energy as the constant allows all foods to be included, not just those with sufficient carbohydrate content, and thus all dietary components and their metabolic interactions can be considered, allowing a more holistic approach to determining insulin demand. Previous studies in healthy people have demonstrated that the FII algorithm is a more accurate predictor of postprandial insulin responses to composite meals than carbohydrate content⁸. A cross-sectional study found no relationship between the FII and glycaemic control in healthy adults, but only fasting biomarkers were considered⁹. In individuals with type 1 diabetes, the FII algorithm was associated with improved postprandial glycaemia without increased risk of

hypoglycaemia compared to carbohydrate counting¹⁰. However, whether the FII algorithm is a better predictor of exogenous insulin requirement in the case of high protein foods with relatively little or no carbohydrate has yet to be investigated. In the present study, our aim was to compare the use of carbohydrate counting versus the FII algorithm for estimating insulin dosage on postprandial glycaemia in adults with type 1 diabetes consuming six commonly consumed protein-containing foods.

4.3 Methods

4.3.1 Study design

We used a triple-blinded, randomised, within-subject crossover design to compare traditional carbohydrate counting with the Food Insulin Index (FII) for estimating insulin dosage on postprandial glycaemia in adults with type 1 diabetes consuming six different single foods. Fifteen adults with type 1 diabetes using insulin pump therapy were recruited through Sydney Insulin Pump Clinic, a private endocrinology clinic in Sydney, Australia. Eligibility criteria included the following: aged between 18 to 70 years, type 1 diabetes diagnosed for ≥ 1 year; use of insulin pump therapy, including proficiency with use of a bolus dose calculator for at least 3 months; HbA1c between 6.0 and 8.5% (42 – 69 mmol/mol); and reliably performing self-monitoring of blood glucose at least four times daily. Exclusion criteria included food allergies, intolerances or eating disorders and use of other medication that may influence blood glucose. The protocol was approved by the Human Research Ethics Committee of the University of Sydney and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000170628). Participants gave written informed consent (See **Appendices 8-12** for participant forms and questionnaires).

Six foods (steak, battered fish, poached eggs, low fat yoghurt, baked beans and salted peanuts) were selected for the study. These were chosen to represent a cross-section of common protein-containing foods with at least a two-fold difference between their carbohydrate content per serving and estimated food insulin demand (FID). FID is the mathematical product of the FII and the energy content (kJ) per normal serving divided by 1000 ($FID = FII \times \text{kJ per serving} / 1000$). For the purposes of this study only, the FID was scaled up by a factor of 100/59 (FID of 1000 kJ of reference food (pure glucose)/g of carbohydrate in 1000 kJ of reference food) so that the existing insulin ratio programmed in the insulin pump could be applied to both algorithms. **Table 4.1** shows the composition of the foods tested including the estimated FID based on the published FII (reference) and energy content per serving.

Each of the 6 single foods was tested in random order on two occasions, once when the insulin requirement was calculated using carbohydrate counting, and once using the estimated FID. Rapid acting insulin was used in both instances using the participants' own insulin pump. A computer-generated randomisation table and sealed envelopes were used so that the order of food and algorithm was randomised, and both researchers and participants were blinded to treatment.

Table 4.1: Nutritional information and serving size for the six test foods

Food	Weight (g)	Energy (kJ)	Fibre (g)	Fat (g)	Protein (g)	CHO* (g)	Avg Insulin Dose using CHO (units)	FID†	Avg Insulin Dose using FID (units)
Beef Steak	225	1350	0	11.4	59.8	0	0.0	31	3.7
Battered Fish	105	945	1.0	14.3	12.4	14	1.6	31	3.7
Poached Egg	180	1080	0	20.3	23.6	2	0.1	15	1.8
Low-fat Strawberry Yoghurt	300	1200	1.2	5.8	13.8	45	5.4	57	6.9
Baked Beans	330	990	16.0	1.9	15.0	36	4.3	49	5.9
Salted Peanuts	150	3900	7.9	78.9	39.5	19	2.4	35	4.2

* CHO, available carbohydrate including sugars and starch and excluding fibre.

† FID, Food Insulin Demand. (FID = FII x kJ in food portion /1000) scaled using the FID and carbohydrate content of 1000 kJ of glucose powder (100/59)).

During the 2 weeks before study commencement, subjects were reviewed by the credentialed diabetes educator (CDE) to optimise insulin basal rates and insulin-to-carbohydrate ratios (ICRs). In the 24 h prior to each testing session, participants were instructed to refrain from the consumption of alcohol and legumes, and any unusual physical activity. On the day of testing, subjects consumed their usual breakfast and lunch meals before presenting to the clinic between 17:00 and 18:00 h. If the BGL was ≥ 10 mmol/L at 15:00 h, the patient administered a correction bolus (normal wave). If the BGL was ≥ 10 mmol/L at the beginning of the testing session, a correction bolus was administered and the subject was asked to return on another day.

At the start of each session, participants were provided with the test food and the designated insulin bolus. The insulin dose was administered as a normal wave bolus immediately prior to the meal by the CDE, who was blinded to the algorithm used to calculate the insulin dose, as well as the test food and subjects BGL during the test session. A dietitian prepared the test food and recorded BGL during the test session but was blinded to the insulin dose administered and the algorithm used. Subjects were blinded to the insulin dose, the algorithm and their BGL during the testing session.

Fingerprick capillary blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min and the glucose concentration determined using a blood glucose monitor (HemoCue, Angelholm, Sweden). If hypoglycaemia occurred (defined as glucose level 3.5 mmol/L or less), the test session was stopped, the event recorded and the patient treated appropriately.

4.3.1 Statistical analysis

A sample size of 15 subjects provided 80% power to detect a 0.5 mmol/L difference in the mean absolute blood glucose level between carbohydrate counting and the FII algorithm, assuming a standard deviation of 0.45 mmol/L. Data were analysed using the SPSS statistical package version 19 (SPSS Inc., Chicago, IL, USA). If the session was stopped due to hypoglycaemia, the last recorded value was carried forward. The primary outcome measure was the mean absolute blood glucose level over 180 min and the secondary measures were: 1) pre-prandial blood glucose level, 2) change in blood glucose over 180 min, 3) peak blood glucose excursion, 4) time to peak blood glucose excursion, 5) mean amplitude of glycaemic excursion (MAGE), 6) time to return to fasting blood glucose level, and 7) number of hypoglycaemia episodes (defined as blood glucose level ≤ 3.5 mmol/L). A general linear model with pre-prandial blood glucose level as a covariate was used to analyse the parameters for the two test algorithms. The number of episodes of hypoglycaemia was expressed as a proportion of all test sessions and compared by Chi-Square test. Differences in coefficients were considered statistically significant if p was < 0.05 , and highly significant if p was < 0.01 (two-tailed). Participant characteristics are presented as mean \pm standard deviation. All other results are presented as mean \pm standard error unless otherwise stated.

4.4 Results

Of the 15 adults recruited, 11 (4 men, 7 women) completed all testing sessions. One participant failed to finish the test sessions due to food ‘intolerance’, another was not available due to family commitments, 1 withdrew following concerns about mild hypoglycaemia and 1 had unstable pre-prandial blood glucose levels on three consecutive

occasions and was excluded from further testing.

The mean age of the 11 subjects who completed the study was 38 years (range: 18-62 years), with a mean BMI of 24.6 ± 2.4 (**Table 4.2**). They had been diagnosed with type 1 diabetes for 14.4 years (range: 2-37 years) and had been using insulin pump therapy for 3.6 years (range: 1-12 years). The mean HbA1c was $6.99 \pm 0.72\%$ (53 ± 7 mmol/mol).

Table 4.2: Subject characteristics for final sample (n=11)

Age (years)	Gender (% Male)	BMI (kg/m ²)	Duration of Diabetes (years)	Duration of Insulin Pump Therapy (years)	HbA1c (% mmol/mol)
38 ± 17 (range: 18-62)	36% (4/11)	24.6 ± 2.4	14.4 ± 14.0 (range: 2-37)	3.4 ± 3.2 (range: 1-12)	7.0 ± 0.7 (53 ± 7)

By chance, baseline (pre-prandial) blood glucose levels were lower when carbohydrate counting was to be used (6.3 ± 0.2 vs 6.9 ± 0.2 mmol/L, $p = 0.04$ for carbohydrate vs FII respectively). Hence, all remaining summary glucose statistics (**Table 4.3**) were adjusted for differences in pre-prandial glucose concentration.

Mean blood glucose levels over 180 min were significantly lower using the FII algorithm compared with carbohydrate counting (5.7 ± 0.2 mmol/L vs 6.5 ± 0.2 mmol/L respectively, $p = 0.003$) (**Table 4.3, FIG 4.1, FIG 4.2**). Mean change in blood glucose level over 3 h was also lower using the FII (-0.7 ± 0.2 mmol/L vs 0.1 ± 0.2 mmol/L, $p = 0.001$).

Peak blood glucose was also reached earlier using the FII algorithm than using carbohydrate counting (34 ± 5 min vs 56 ± 7 min, $p = 0.007$). Peak change in blood glucose level from baseline tended to be lower when using the FII algorithm compared to carbohydrate counting, although the difference was not significant (1.3 ± 0.2 mmol/L vs 1.8 ± 0.3 mmol/L respectively, $p = 0.13$). In contrast, the maximum amplitude of the glycaemic excursion (MAGE = the difference between the maximum and minimum observed blood glucose values) was significantly larger using the FII algorithm than carbohydrate counting (4.4 ± 0.2 mmol/L vs 3.7 ± 0.2 mmol/L, $p = 0.02$).

Mild hypoglycaemia requiring cessation of the session per protocol occurred frequently under both conditions and across all foods (**FIG 4.2**), but there was no difference between the two algorithms (hypoglycaemia occurred in 48% vs 33% of all test sessions for FII vs carbohydrate counting respectively, $p = 0.155$).

Table 4.3: Mean results for pre-prandial blood glucose, postprandial blood glucose, change in blood glucose and peak blood glucose level and the actual number of hypoglycaemic episodes for test food and algorithm.

Food	Algorithm	Pre-prandial blood glucose level (mmol/L)	Mean blood glucose level (mmol/L)	Mean change in blood glucose level (mmol/L)	Peak change blood glucose level (mmol/L)	Number of episodes of hypoglycaemia (≤ 3.5 mmol/L)
Beef Steak	FII	7.2 ± 0.5	4.6 ± 0.4	- 1.7 ± 0.4	0.3 ± 0.2	8 / 11
	CC	6.5 ± 0.4	6.7 ± 0.4	0.2 ± 0.4	2.4 ± 0.9	2 / 11
Battered Fish	FII	6.5 ± 0.4	5.8 ± 0.4	- 0.5 ± 0.4	0.9 ± 0.2	5 / 11
	CC	5.8 ± 0.4	6.9 ± 0.4	0.7 ± 0.4	2.5 ± 0.5	3 / 11
Poached Eggs	FII	7.4 ± 0.6	5.2 ± 0.4	- 1.5 ± 0.4	0.7 ± 0.3	5 / 11
	CC	6.8 ± 0.6	5.7 ± 0.4	- 0.9 ± 0.4	0.6 ± 0.4	2 / 11
Strawberry Yoghurt	FII	7.2 ± 0.6	6.1 ± 0.4	- 0.5 ± 0.4	1.7 ± 0.5	6 / 11
	CC	6.3 ± 0.4	6.9 ± 0.4	0.6 ± 0.4	2.3 ± 0.6	4 / 11
Baked Beans	FII	6.2 ± 0.6	7.3 ± 0.4	0.9 ± 0.4	3.4 ± 0.7	3 / 11
	CC	6.3 ± 0.3	7.0 ± 0.4	0.5 ± 0.4	2.6 ± 0.5	5 / 11
Salted Peanuts	FII	7.1 ± 0.6	5.4 ± 0.4	- 1.1 ± 0.4	0.9 ± 0.4	5 / 11
	CC	6.3 ± 0.4	5.4 ± 0.4	- 0.2 ± 0.4	0.4 ± 0.2	6 / 11
Overall	FII	6.9 ± 0.5	5.7 ± 0.2	-0.7 ± 0.2	1.3 ± 0.2	32 / 66
	CC	6.3 ± 0.4	6.5 ± 0.2	0.1 ± 0.2	1.8 ± 0.3	22 / 66
P value		0.039	0.003	0.001	0.835	0.155

Data presented as mean ± SE, with the exception of the episodes of hypoglycaemia, which is presented as the actual number. N = 11 adults subjects with type 1 diabetes using insulin pump therapy. CC = Carbohydrate Counting.

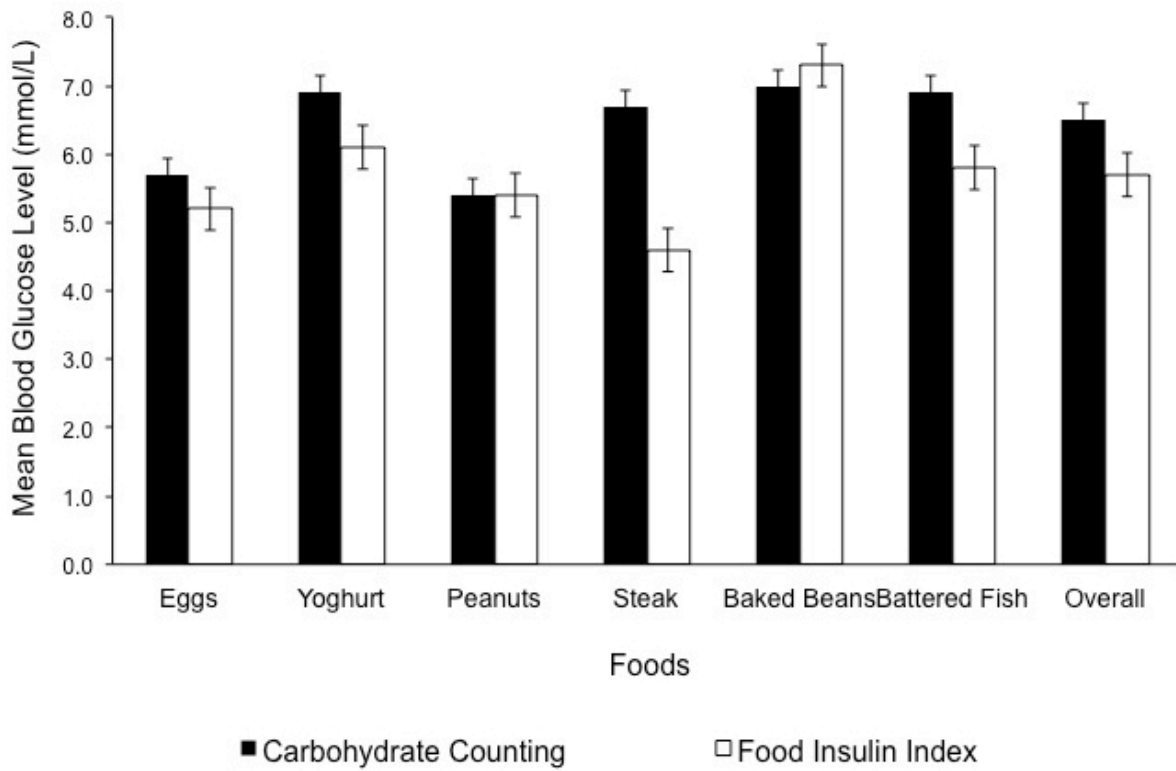


Figure 4.1: Mean \pm SEM blood glucose level after consumption of 6 foods in 11 subjects with type 1 diabetes

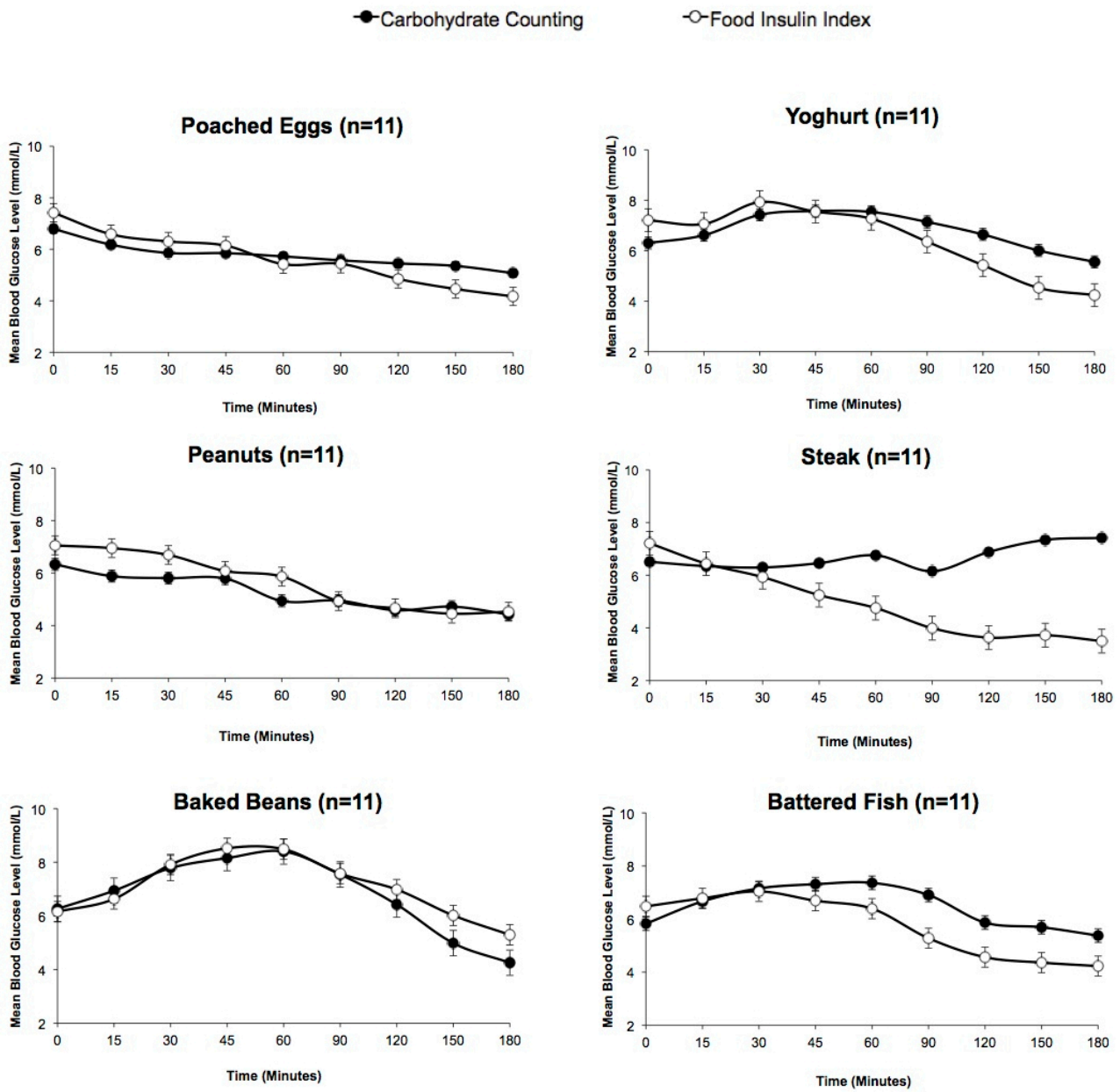


Figure 4.2: Changes in blood glucose after consuming protein-containing foods using either carbohydrate counting or the food insulin index.

4.5 Discussion

The current study demonstrates that the application of the FII to single, protein-containing foods improves postprandial hyperglycaemia in comparison to traditional carbohydrate counting. Over the 3 h monitoring period, the FII algorithm reduced the mean blood glucose level by an average of ~12%, and reduced the time to reach peak blood glucose concentration by almost half. However, MAGE was higher using the FII compared with carbohydrate counting and both treatments were associated with relatively high rates of mild hypoglycaemia. Postprandial hyperglycaemia is common in everyday life and associated with increased risk of developing cardiovascular disease, nephropathy, retinopathy and neuropathy¹¹.

For this study, we specifically chose protein-containing foods where apparent insulin demand was at least 2-fold higher according to the FII as compared to carbohydrate counting. Indeed, two of the test foods (steak and eggs) contained little or no carbohydrate. We therefore expected to find more hypoglycaemia using the FII. However, hypoglycaemia (defined as BGL < 3.5 mmol/L occurred frequently under both conditions and across all foods, with no statistical difference between the two algorithms (48% vs 33% for FII vs carbohydrate counting respectively, $p = 0.155$). The observed trend towards increased hypoglycaemia with the FII seen in this study warrants further investigation given the risks of hypoglycaemia in contributing to oxidative stress and diabetes complications^{12,13}. Potentially, the normal wave bolus does not closely follow the normal postprandial physiological insulin profile for high protein and/or fat foods, with excess insulin delivered initially resulting in increased risk of hypoglycaemia. Previous research has highlighted the benefit of dual-wave or square insulin bolus delivery patterns in these instances as well as for low GI meals, as a portion of the total insulin dose is delivered immediately (often 50-70%) and the remainder delivered slowly over

an extended period of time¹⁴⁻¹⁷. Further research to elucidate the optimal bolus insulin pattern for foods of varying FII as well as macronutrient content is warranted.

Although carbohydrate counting is currently considered the gold standard in determining mealtime insulin dose, it is essentially focused on treating the symptom of type 1 diabetes (hyperglycaemia) rather than the underlying cause – endogenous insulin insufficiency. As an anabolic hormone, insulin plays an important role in the metabolism of all three macronutrients: carbohydrate, protein and fat. In healthy subjects, protein elicits a similar insulin response to the same amount of glucose in many slowly digested carbohydrate foods¹⁸. Fat and protein also increase hepatic glucose output and acute insulin resistance^{5, 19-21}, which may help explain the increased insulin demand for foods rich in these macronutrients and why hypoglycaemia was not seen in every test session. In addition, fat and protein also slow gastric emptying and thereby reduce postprandial glycaemia compared with meals containing little protein and/or fat^{22, 23}. However, carbohydrate counting entirely ignores the presence of fat and protein in foods because neither directly contributes to glycaemia. Our present findings suggest that the FII, based on actual insulin responses in healthy subjects, may be a more reliable predictor of insulin dose in those with type 1 diabetes consuming protein-containing meals.

The strengths of this study include the randomised, controlled design in which researchers, participants and technician were blinded to treatment. This reduces the risk of bias and improves the reliability of the findings. This study is also the first to explore the concept of food insulin demand ($FID = FII \times \text{kJ per serving}/1000$), a formula for translating the relative FII values into units proportional to the food portion size. This is a critical step towards applying the FII algorithm in clinical practice. The FID allows a mealtime insulin dose to be calculated based on the FII of the food (or foods) to be consumed and the actual portion size.

For the purposes of this study, the food insulin demand was scaled so that the FID value could be imputed into the insulin pump without the need to change the subject's existing individualised carbohydrate: insulin ratio.

The study has limitations. Although subjects were instructed to consume their usual breakfast and lunch meals at the same time on each testing day, these meals were not standardised and therefore there may have been a confounder.

Test sessions where the subject experienced hypoglycaemia ($BGL < 3.5$ mmol/L) were terminated immediately so the hypoglycaemia could be treated. In these cases, the last recorded blood glucose level was carried forward. Alternately, we could have chosen to assume a consistent gradient in BGLs over the remainder of the recording period. However, imputing any value for missing data raises doubts about the generalisability of the findings. It could also be argued that 3 h recordings are not sufficiently long enough to detect delayed effects of an insulin dose. The efficacy of the FII algorithm over a longer time period should be the subject of further research. Additionally, the study was powered to compare mean blood glucose concentrations, rather than prevalence of hypoglycaemia. The lack of difference in hypoglycaemia may therefore be due to insufficient power. Further studies with a larger sample size are needed.

Despite these limitations, this study adds to a growing body of evidence supporting the use of the novel FII algorithm as a promising tool for predicting prandial insulin dose in adults with type 1 diabetes.

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CHAPTER 5

Clinical application of the Food Insulin Index

in the diet of adults with type 1 diabetes:

The FOODII Study

5.1 Abstract

Objective: The Food Insulin Index (FII) is a novel algorithm for ranking foods based on their insulin demand relative to an isoenergetic reference food. We compared carbohydrate counting versus the FII algorithm for estimating insulin dosage on changes in glycated haemoglobin A1c and postprandial glycaemia over 12 weeks in adults with type 1 diabetes.

Methods: In a randomised, controlled parallel study design, adults (n = 26) using insulin pump therapy were assigned to either traditional carbohydrate counting (CC) or the novel FII. Subjects participated in group education and individual sessions with a research dietitian. Written resources, a smartphone app, email and telephone support were offered. At baseline and on completion of the trial, glycated haemoglobin, day-long glycaemia (6-day continuous glucose monitoring), fasting lipids and C-reactive protein were determined.

Results: Changes in HbA1c from baseline to 12 weeks were similar in both groups (mean \pm SEM: FII: $-0.1 \pm 0.1\%$ vs CC: $-0.3 \pm 0.2\%$, $p = 0.855$). Both groups spent a similar amount of time within the normal glycaemic range (FII: $42 \pm 18\%$ vs CC: $39 \pm 18\%$, $p = 0.646$) and hyperglycaemic range (FII: 50.9 ± 6.4 vs CC: 54.6 ± 5.0 , $p = 0.814$). Only the FII counters experienced a trend (-43% , $p = 0.057$) to reduced hypoglycaemia at 12 weeks.

Conclusions: In a 12-week pilot study, changes in glycated haemoglobin and postprandial glycaemia were similar using FII counting or carbohydrate counting. A trend to reduced risk of hypoglycaemia in FII counters warrants further study.

5.2 Introduction

Achieving optimal postprandial glycaemic control is an important aspect of management in type 1 diabetes to minimise the risk of acute and chronic complications. Yet despite significant advancements in insulin therapy, optimum postprandial glycaemic control remains difficult to achieve. Even patients within target glycated haemoglobin A1c (HbA1c) levels continue to experience unanticipated hyper- and hypoglycaemia, particularly in response to meals high in protein and/or fat¹⁻³.

Carbohydrate counting is frequently recommended as routine therapy for patients with type 1 diabetes. However numerous studies in healthy subjects have demonstrated that the same amount of carbohydrate from different food sources produces wide variations in blood glucose and insulin responses³⁻⁶. Furthermore, studies in vitro and in vivo have demonstrated the significant role of protein and fat in addition to carbohydrate on normal physiological insulin secretion⁷⁻⁹. A more comprehensive understanding of the relationship between dietary factors and physiological insulin secretion evoked by foods is likely to improve clinical and practical outcomes in the management of type 1 diabetes.

The Food Insulin Index (FII) is a novel algorithm of ranking foods based on the insulin response ('demand') in healthy subjects relative to an isoenergetic reference food¹⁰. The algorithm uses food energy as the constant and thus all dietary components and their metabolic interactions can be considered for all foods with sufficient energy density, allowing a holistic approach to determining insulin demand. Previous studies in healthy people have demonstrated that the FII algorithm is a more accurate predictor of observed insulin responses to composite meals than carbohydrate content¹¹. In individuals with type 1 diabetes, the FII algorithm was associated with improved postprandial glycaemia without increased risk of

hypoglycaemia compared to carbohydrate counting¹². The FII algorithm can theoretically be integrated into clinical practice by counting ‘units’ of insulin demand per serving in the same way that carbohydrate is counted. The food insulin demand (FID) is a formula for translating the relative FII values into units proportional to the food portion’s energy content ($FID = FII \times \text{kJ per serving} / 1000$). The FID allows a mealtime insulin dose to be calculated based on the FII of the food (or foods) to be consumed and the actual portion size. Prandial insulin doses are then determined using an individualised insulin: FID ratio akin to that applied when using the insulin: carbohydrate ratio. However, whether estimating prandial insulin doses using the FII algorithm and FID counting is feasible in practice and is beneficial for glycaemic control has not been investigated. Therefore in the present study, our aim was to compare the use of carbohydrate counting versus the FII algorithm for estimating mealtime insulin dosage on postprandial glycaemia in adults with type 1 diabetes over 12 weeks. This was the first study to apply the FII to a chronic feeding situation.

5.3 Methods

5.3.1 Study Design

In this randomised, controlled, parallel design pilot study, we compared traditional carbohydrate counting with the FII for estimating mealtime insulin dosage on postprandial glycaemia in adults with type 1 diabetes over 12 weeks. Twenty-six adults with type 1 diabetes using insulin pump therapy were recruited through Sydney Insulin Pump Clinic, a private endocrinology clinic in Sydney, Australia. Eligibility criteria included the following: aged between 18 to 70 years, type 1 diabetes diagnosed for ≥ 1 year; use of insulin pump therapy, including proficiency with use of a bolus dose calculator for at least 3 months; HbA1c between 7.0 and 9.5% (53 – 80 mmol/mol); and reliably performing self-monitoring

of blood glucose at least four times daily. Suboptimal glycaemic control, assessed by HbA1c, was required for eligibility as this study aimed to improve glycaemic control. Exclusion criteria included food allergies, intolerances or eating disorders and use of other medication that may influence blood glucose. The protocol was approved by the Human Research Ethics Committee of the University of Sydney and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613001367730). See **Appendices 13 – 17** for participant forms and questionnaires.

Subjects were randomly allocated one of two methods of estimating their mealtime insulin doses: traditional carbohydrate counting (CC) or the FII. Randomisation was achieved through a computer-generated randomisation table and the use of opaque sealed envelopes. The allocation sequence was concealed from patients and investigators until the commencement of the trial.

Subjects participated in a 2 h interactive group education workshop with the research dietitian. This workshop covered the principles of the allocated method, awareness of foods to be counted and orientation to the carbohydrate or FID values of common foods and instruction on calculating mealtime insulin doses. Participants were given workshop manuals, pictorial booklets, pocket-sized guides and given access to a website and a smartphone app to assist with their ‘counting’ and dose calculations. These resources were all designed *de novo* by the candidate in order to be as similar as possible with only key information and numerical food values being different. For illustrative purposes, sample copies of the materials are included at the end of this chapter and the session plans are included in **Appendix 18**. For foods not included in the materials, participants in both groups were instructed to use the value of a similar food and were encouraged to contact the research dietitian if assistance was required.

During the first education session, subjects were asked to record a 24 h food recall and identify the carbohydrate content or FID of their meals and snacks using the resources provided and practice estimating the portion sizes and corresponding carbohydrate content/FID of self-served common, real foods, such as rice, pasta, milk, juice, cereal and in the case of FID counters – meats. At the conclusion of the workshop the FID counters had their individualised ICR converted to an insulin: FID ratio (IFR), to allow the calculation of insulin doses. The IFR was calculated by scaling the usual ICR by a factor of 1.7 ($1.7 = 100$ divided by $59 =$ FII of 1000 kJ of glucose divided by grams of carbohydrate in 1000 kJ of glucose). For example, an ICR of 1 unit: 10 g carbohydrate became an IFR of 1 unit: 17 FID. In this way, the IFR automatically adjusted for differences in insulin sensitivity among subjects. No changes were made to the ICR of the carbohydrate counters. Telephone and email support was offered to both groups and all participants received a follow-up email each week throughout their intervention.

In the week following their group education workshop, subjects completed a food diary and recorded their carbohydrate/FID estimations and blood glucose levels. At the end of the week, they attended a 30 min follow-up individual appointment with the research dietitian to review their food diary and estimations, discuss estimations for other food preferences and individualise the information provided in the group workshop.

During the week prior to the study and then again after the 12 week intervention, subjects wore a continuous glucose monitoring system (CGMS; iProTM2, Medtronic) for 6 consecutive days to capture glycaemic control. HbA1c, blood lipids and C-reactive protein (CRP) were measured at baseline and end of the study to assess average glycaemic control, lipid metabolism and low grade chronic inflammation.

5.3.2 Statistical analysis

A sample size of 38 subjects was calculated to provide 80% power to detect a 0.3% point difference in HbA1c between carbohydrate and FID counting, assuming a standard deviation of 0.32 mmol/L. Interim analysis after 25 subjects had completed the trial revealed no clinically or statistically significant difference in HbA1c between the groups. The pilot study was terminated at this point. Data were analysed using the SPSS statistical package version 21 (SPSS Inc., Chicago, IL, USA). Changes in the following parameters between baseline and 12 weeks were analysed within and between groups: 1) glycated haemoglobin A1c (HbA1c), 2) mean blood glucose levels, 3) percentage of time within low (< 3.9 mmol/L), normal (3.9 – 7.8 mmol/L) and high (>7.8 mmol/L) blood glucose ranges, 4) mean amplitude of glycaemic excursions (MAGE) and 5) number of hyperglycaemic (>7.8 mmol/L) and hypoglycaemic (< 3.9 mmol/L) episodes. Differences in coefficients were considered statistically significant if p was < 0.05, and highly significant if p was < 0.01 (two-tailed). For subjects who withdrew, available baseline measures were carried forward for analysis. Participant characteristics are presented as mean \pm standard deviation. All other results are presented as mean \pm standard error unless otherwise stated.

5.4 Results

A total of 26 adults responded to recruitment notices and met the inclusion criteria. Of these, 14 were randomised to FID counting and 12 to CC (**FIG. 5.1**). Twenty-two subjects completed all testing sessions (10 men, 12 women), with 4 subjects withdrawing due to work commitments (two participants withdrew prior to completing all baseline measures and therefore could not be included in analyses, and two withdrawing following the group education session. In this case, baseline measures were carried forward to 12 weeks).

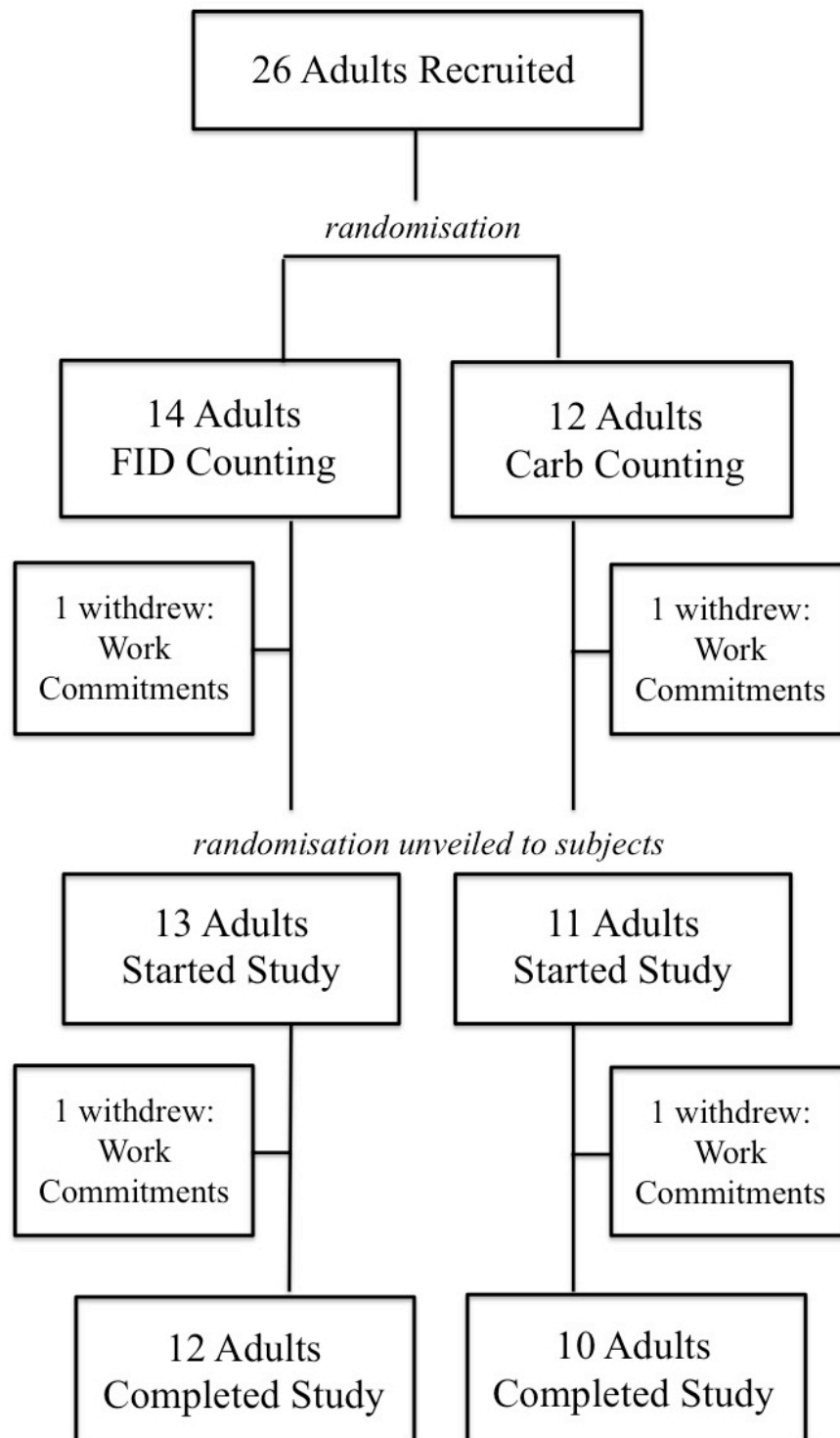


Figure 5.1: Flow of subjects through the study.

Subject characteristics are shown in **Table 5.1**. Mean age, BMI and baseline HbA1c were similar in each group.

Table 5.1: Participant characteristics

	Carbohydrate counters (n = 12)	FID counters (n = 14)
Gender (% male)	58	36
Age (years)	41 ± 18 (range: 20 – 67)	38 ± 15 (range: 23 – 68)
BMI (kg/m²)	25.9 ± 2.3	26.6 ± 7.0
Baseline HbA1c	8.6 ± 0.3	8.1 ± 0.2
(% (mmol/mol))	(70 ± 3)	(65 ± 2)
Baseline fasting BG (mmol/L)*	6.9 ± 1.8	8.2 ± 3.4

* CC: n = 11, FID: n = 13

The main findings are summarised in **Table 5.2**. Changes in HbA1c after 12 weeks of intervention were not different between the groups (FID $-0.1 \pm 0.1\%$ vs CC $-0.3 \pm 0.2\%$, $p = 0.855$) (**FIG 5.2**). Both groups spent a similar amount of time within the normal glycaemic range (FID $42 \pm 18\%$ vs CC $39 \pm 18\%$, $p = 0.646$) (**FIG 5.3**). Both groups spent about the same time in the hyperglycaemic range (FID: 50.9 ± 6.4 vs CC: 54.6 ± 5.0 , $p = 0.814$). FID counters experienced a trend to reduced time in the hypoglycaemic range ($4 \pm 4\%$ at 12 weeks vs $7 \pm 5\%$ at baseline, $p = 0.057$) while the CC group showed no change at end of the trial ($10 \pm 7\%$ at 12 weeks vs $9 \pm 6\%$ at baseline $p = 0.684$). Changes in lipids or CRP were similar in both groups.

Table 5.2: Mean results for HbA1c, fasting blood glucose, mean blood glucose, proportion of time in low, normal and high blood glucose ranges, number of low and high blood glucose events, total, LDL and HDL cholesterol, triglycerides and C-reactive protein for carbohydrate counters and FID counters at baseline and 12 weeks.

	Carbohydrate Counters (n =11)				FID Counters (n=13)				Between Groups P Values		
	<i>Baseline</i>	<i>12 weeks</i>	<i>Change</i>	<i>P Value</i>	<i>Baseline</i>	<i>12 weeks</i>	<i>Change</i>	<i>P Value</i>	<i>Baseline</i>	<i>12 Weeks</i>	<i>Change</i>
HbA1c (% (mmol/mol))*	8.6 ± 0.3;	8.3 ± 0.2;	-0.3 ± 0.2	0.193	8.1± 0.2;	8.0 ± 0.2;	-0.1 ± 0.1	0.921	0.112	0.236	0.308
	70 ± 3	67 ± 2			65 ± 2	64 ± 2					
Fasting BG (mmol/L)	6.9 ± 0.6	7.1 ± 0.4	0.2 ± 0.6	0.784	8.2 ± 0.9	8.2 ± 0.4	0.0 ± 0.8	0.979	0.273	0.063	0.886
Mean BG Level (mmol/L)	9.1 ± 0.4	8.8 ± 0.6	-0.2 ± 0.6	0.682	8.9 ± 0.4	8.8 ± 0.4	-0.2 ± 0.5	0.771	0.819	0.954	0.907

	Carbohydrate Counters (n =11)				FID Counters (n=13)				Between Groups P Values		
	<i>Baseline</i>	<i>12 weeks</i>	<i>Change</i>	<i>P Value</i>	<i>Baseline</i>	<i>12 weeks</i>	<i>Change</i>	<i>P Value</i>	<i>Baseline</i>	<i>12 Weeks</i>	<i>Change</i>
Time in low BG range (%)	8.5 ± 1.8	9.4 ± 2.1	0.8 ± 1.9	0.682	7.1 ± 1.4	4.0 ± 1.1	-3.1 ± 1.5	0.058	0.523	0.029	0.117
Time in normal BG range (%)	35.5 ± 2.8	39.7 ± 5.0	4.3 ± 4.6	0.378	43.0 ± 5.6	41.4 ± 4.8	-1.6 ± 7.5	0.833	0.264	0.814	0.529
Time in high BG range (%)	56.0 ± 4.1	50.9 ± 6.4	-5.1 ± 5.4	0.371	50.0 ± 5.9	54.6 ± 5.0	4.7 ± 8.2	0.575	0.425	0.646	0.347
# of Low BG Events	8.7 ± 1.6	7.9 ± 1.5	-0.8 ± 1.5	0.585	6.0 ± 1.2	4.6 ± 1.1	-1.4 ± 1.3	0.314	0.179	0.091	0.775
# of High BG Events	17.6 ± 1.1	13.9 ± 1.3	-3.7 ± 1.9	0.079	14.7 ± 1.0	16.2 ± 1.3	1.5 ± 1.6	0.381	0.066	0.229	0.048

	Carbohydrate Counters (n =11)				FID Counters (n=13)				Between Groups P Values		
	Baseline	12 weeks	Change	P Value	Baseline	12 weeks	Change	P Value	Baseline	12 Weeks	Change
Total Chol. (mmol/L)	4.4 ± 0.2	4.5 ± 0.2	0.1 ± 0.1	0.404	4.7 ± 0.2	4.9 ± 0.3	0.2 ± 0.3	0.503	0.270	0.347	0.733
HDL-Chol. (mmol/L)	1.7 ± 0.1	1.7 ± 0.1	0	0.555	1.5 ± 0.1	1.5 ± 0.1	0	0.221	0.193	0.150	0.663
LDL-Chol. (mmol/L)	2.4 ± 0.2	2.5 ± 0.2	0.1 ± 0.1	0.376	2.6 ± 0.2	2.8 ± 0.3	0.2 ± 0.2	0.291	0.616	0.364	0.551
Triglycerides (mmol/L)	0.7 ± 0.0	0.7 ± 0.1	0	0.601	1.5 ± 0.3	1.3 ± 0.3	-0.2 ± 0.3	0.608	0.011	0.042	0.570
CRP (mg/L)	1.6 ± 0.5	0.9 ± 0.2	-0.6 ± 0.4	0.173	6.3 ± 2.7	3.7 ± 1.7	0.4 ± 1.2	0.741	0.090	0.153	0.413

* CC: n = 12, FID: n = 14; BG = Blood Glucose Level; Chol. = cholesterol; CRP = C-Reactive Protein; Low BG = (< 3.9 mmol/L); Normal = 3.9 – 7.8

mmol/L; High = > 7.8 mmol/L.

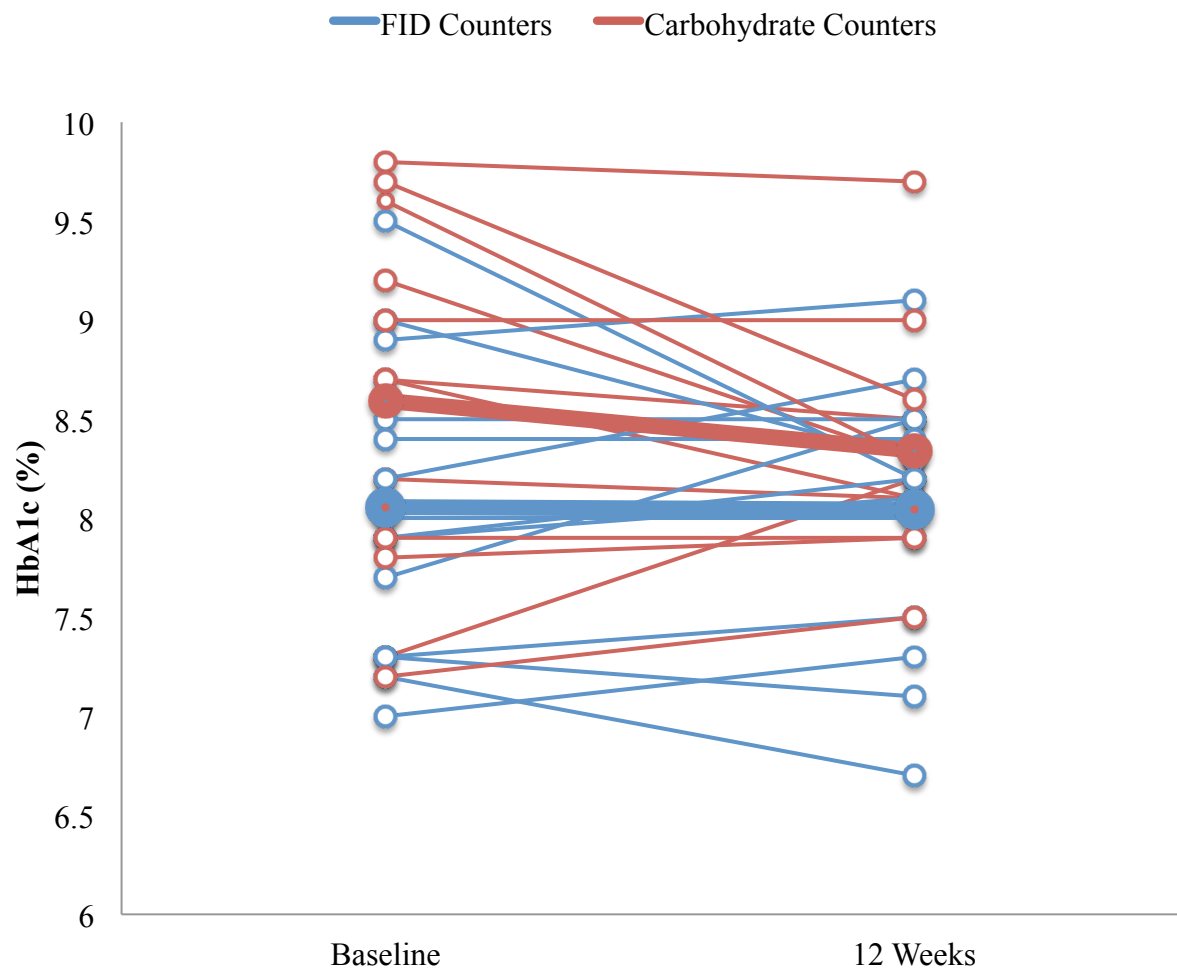


Figure 5.2: Changes in HbA1c over 12 weeks in adults with type 1 diabetes using either FID counting (n = 14) or traditional carbohydrate counting (n = 12) for estimating mealtime insulin doses (Group means in bold).

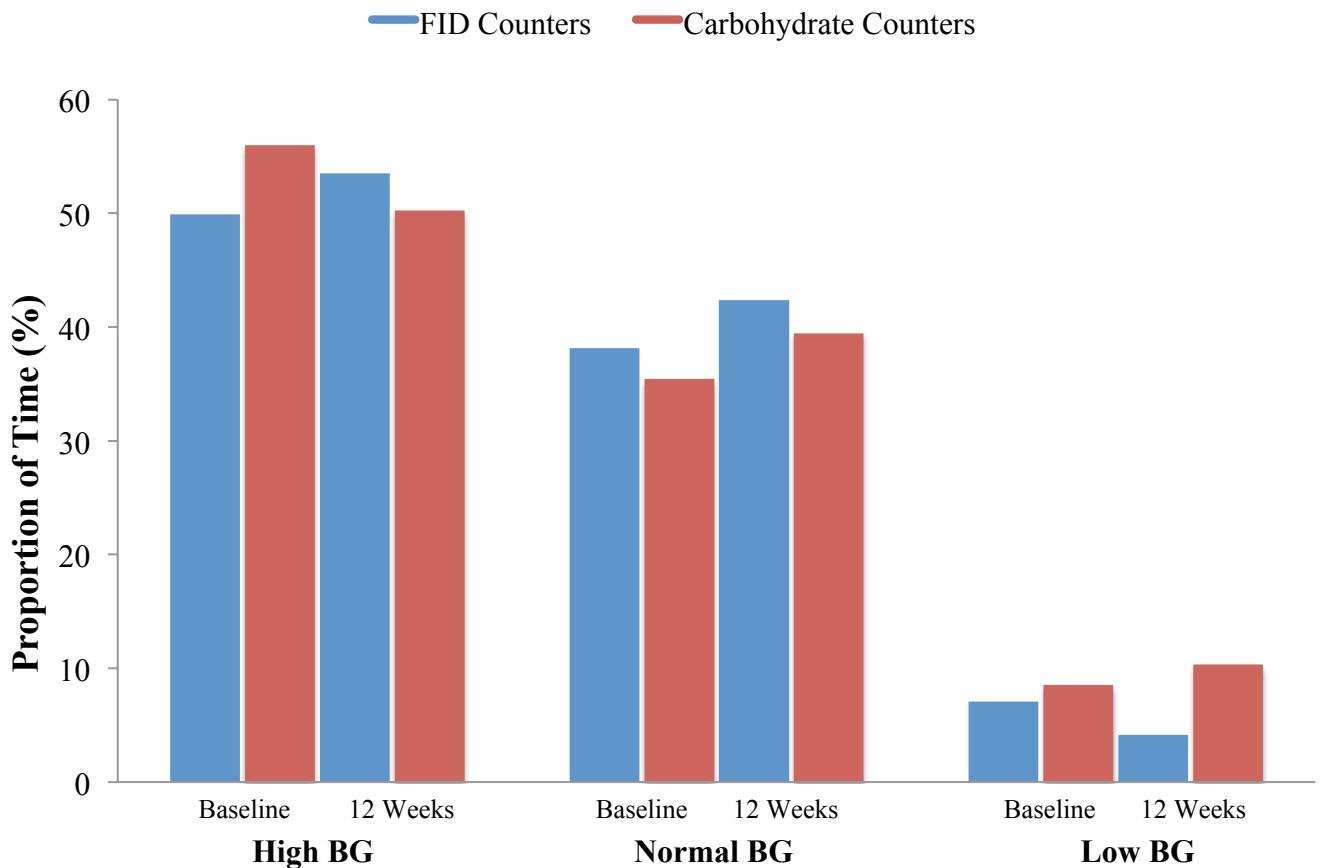


Figure 5.3: Proportion of time spent in high, normal and low BG ranges for adults with type 1 diabetes using either traditional carbohydrate counting or FID counting.

When asked to rate their experience with their allocated insulin dosing method, participants in both groups agreed that their method was easy to use and that they were able to enjoy a wide range of foods. Approximately half of the participants in both groups felt their blood glucose levels were better managed during the study (FID: 54% vs CC: 55%), while the remainder felt there had been no change. None of the subjects felt their glycaemic control had deteriorated while they were involved in the study. In the FID group, 46% of subjects stated that they would continue using FID counting to estimate their bolus insulin dose.

5.5 Discussion

This is the first study of the clinical application of the FII to estimate insulin bolus requirements in adults with type 1 diabetes. The findings indicate that FID counting was at least as good as carbohydrate counting for maintaining glycaemic control in adults with type 1 diabetes using insulin pump therapy. Changes in HbA1c, mean blood glucose and the time within the optimum glycaemic range were comparable in both groups. The number of hyperglycaemic episodes was similar, but FID counters showed a trend to reduced risk of hypoglycaemia (-43%, $p = 0.058$) after using the FII for 12 weeks, inferring the potential for improved glycaemic stability.

There are surprisingly few randomised, controlled trials assessing the efficacy of carbohydrate counting for glycaemic control in type 1 diabetes. Our recent meta-analysis showed that carbohydrate counting did not result in significant improvements in HbA1c when compared with routine care or an alternative strategy¹³. Schmidt et al recently conducted a 16-week study comparing carbohydrate counting intervention with usual care¹⁴. Although they achieved a large reduction in HbA1c in their carbohydrate counting group (-0.8% points), this improvement was not significantly different to that of their control group ($p = 0.175$). Two other trials have found other strategies, such as flexible low GI education, were more effective than carbohydrate counting^{12, 15}.

One of the strengths of the present study is the provision of an 'attention placebo', ie a control group who received a similar intensity of interaction as the intervention group. Since glycaemic control can improve due to increased contact time with healthcare professionals, both groups received identical attention throughout the trial. Subjects in both groups attended similarly structured group and individual appointments with the research dietitian, received identical resources, with only the key information and numerical food values changed and

were offered the same telephone and email support throughout the trial. This attention placebo ensures that the differences in HbA1c observed were a direct result of the method of estimating mealtime insulin dose employed.

This study was designed as a feasibility study to test whether FID counting could be used in practice in type 1 diabetes. It has been suggested that the FID counting is too complex to be used in practice, however our experience during this study revealed this was not the reality. All participants using FID counting appeared to adapt quickly to the new algorithm. At the conclusion of the trial, all rated the method as “easy to use”, with just under half of subjects opting to continue with the system over carbohydrate counting if the option were available. Two subjects withdrew from the study following the initial group education workshop, however they had been allocated to different arms of the study, suggesting this was not due to their intervention allocation.

There are several limitations to this study. It was designed as a pilot study with a small sample size in order to keep a close eye on participants and assess the feasibility of the FII algorithm in clinical practice and to gauge the sample size necessary for a larger trial. The study was therefore not powered to detect clinically important differences in HbA1c, however, the observed difference in HbA1c does not suggest any important advantage of one strategy over the other. Based on the standard deviation in HbA1c of 0.57 seen in this study (average SD of both groups), 116 subjects would be required to achieve 80% power to detect a difference of 0.3% points in HbA1c.

The potential of the FII to reduce the risk of hypoglycaemia is an exciting prospect that was not foreseen and thus the study was not powered to detect differences in the risk of hypoglycaemia. This finding contrasts to that of previous studies, including Chapter 4 of this thesis, which revealed a trend towards an increased risk of hypoglycaemia with the FII,

though neither were statistically significant^{12,15}. Potentially the risk of hypoglycaemia is reversed in practice and therefore future studies with sufficient power are warranted. To detect the same degree of improvement in hypoglycaemia (~45% reduction), 90 subjects are needed. However, to detect a smaller but clinically important 25% reduction in rate of hypoglycaemia, 180 subjects would be required.

The potential for bias was real because the research dietitian was responsible for both patient instruction as well as assessment. However, the primary outcome was biochemical and collected and assessed by external laboratories who were blinded to treatment. It is therefore unlikely that the main results were influenced by detection bias.

Carbohydrate counting instruction is a routine part of clinical management of type 1 diabetes in Australia and is a compulsory requirement of initiating insulin pump therapy. This gave the carbohydrate counters a distinct advantage because they were all familiar and proficient with this algorithm. The FID counters, however, were required to learn and master the novel strategy in the space of a few weeks. This need for adjustment may have been a confounder that attenuated any potential improvement in HbA1c. In addition, the insulin dose to food ratio in the FII counters was simply extrapolated from their existing ICR, rather than determined through titration according to normal practice. Hence, the design of future studies should consider targeting newly diagnosed subjects with type 1 diabetes who have no prior knowledge of carbohydrate counting. In this scenario, new patients' educators can titrate their own insulin to food ratio according to the degree of hyper and hypoglycaemia experienced. Future studies should also be conducted for a longer duration, with frequent contact with health professionals, especially initially to assist with the mastery of the new food counting system and the titration of the mealtime insulin ratio. Collection of insulin therapy data (e.g. insulin pump downloads) and detailed food diaries will also provide further insights into

study compliance and postprandial blood glucose control. These considerations will also be important for the implementation of FID counting, or any other new system of estimating insulin dose in clinical practice.

This pilot study also highlighted other issues that should be resolved before undertaking a larger clinical trial. Some subjects commented that the limited number of foods with a tested FII value made some meal estimations difficult. This is an inherent limitation of FID counting at present as the FII of each food is determined from the postprandial insulin responses of 10 lean, healthy adults and cannot be calculated from the macronutrient composition. At the time of the study the FII of 127 foods had been tested, representing a broad cross-section of commonly eaten foods in the Western diet. However, expansion of the FII database is ongoing and needs to continue before FID counting could be widely implemented.

In conclusion, changes in HbA1c and postprandial glycaemia were similar using FII counting or carbohydrate counting in a 12-week pilot study. The near-significant trend to reduced risk of hypoglycaemia in FII counters warrants further study.


5.6 References

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RESOURCES

Excerpts from the pocket-sized guides




The FOODII Study

**FID Counter:
Pocket-Sized
Guide**

Dairy Products		
Food	Serve Size	FID
Skim Milk	250mL	23
Full Cream Milk	250mL	17
Low Fat Fruit Yoghurt	175g Tub	57
Vanilla Ice-Cream	1 Scoop (50g)	27
Low Fat Vanilla Ice-Cream	1 Scoop (50g)	19
Fruit Frozen Yoghurt	1 Scoop (50g)	18
Cheddar Cheese	1 Slice (25g)	14
Low Fat Cheddar Cheese	1 Slice (21g)	4
Low Fat Processed Cheese	1 Slice (20.5g)	6
Cream Cheese	1 Tablespoon (20g)	5
Reduced Fat Cottage Cheese	½ Cup (120g)	21
Low Fat Cottage Cheese	½ Cup (120g)	24

9



The FOODII Study

**Carb Counter:
Pocket-Sized
Guide**

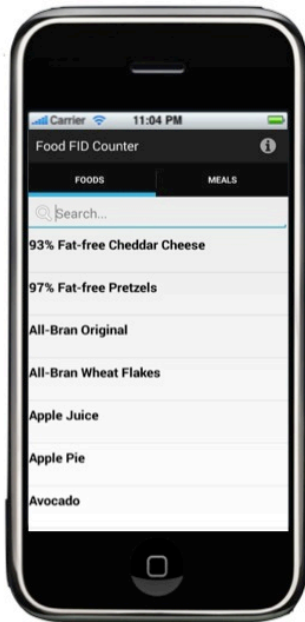
Dairy Products		
Food	Serve Size	Carbs (g)
Skim Milk	250mL	13
Full Cream Milk	250mL	13
Low Fat Fruit Yoghurt	175g Tub	26
Vanilla Ice-Cream	1 Scoop (50g)	11
Low Fat Vanilla Ice-Cream	1 Scoop (50g)	12
Fruit Frozen Yoghurt	1 Scoop (50g)	11
Cheddar Cheese	1 Slice (25g)	0
Low Fat Cheddar Cheese	1 Slice (21g)	0
Low Fat Processed Cheese	1 Slice (20.5g)	2
Cream Cheese	1 Tablespoon (20g)	1
Reduced Fat Cottage Cheese	½ Cup (120g)	4
Low Fat Cottage Cheese	½ Cup (120g)	7

9

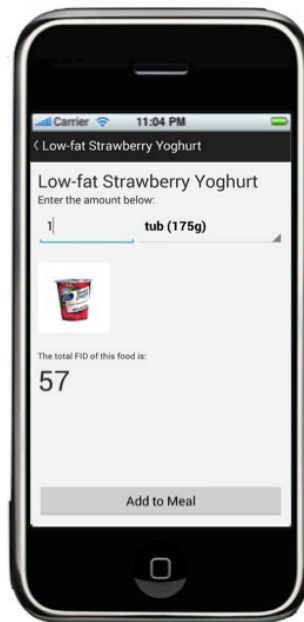
Screenshots of the Smartphone Apps

FID Counter

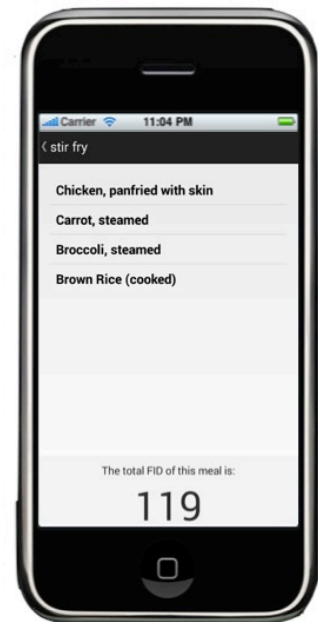
A: Searchable Food List



B: FID of Selected Food and Portion Size

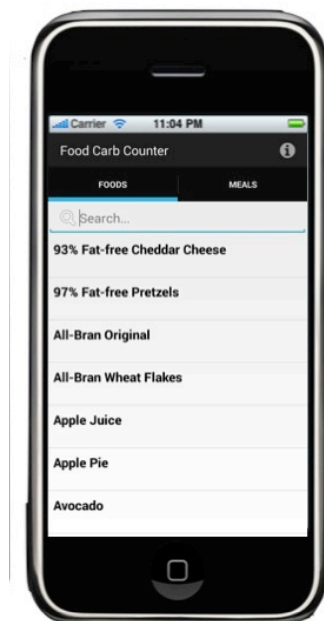


C: FID of Entered Recipe/Meal

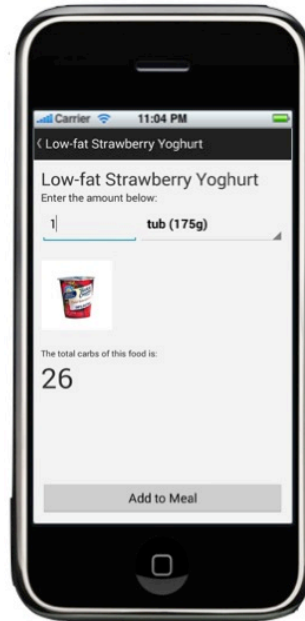


Carb Counter

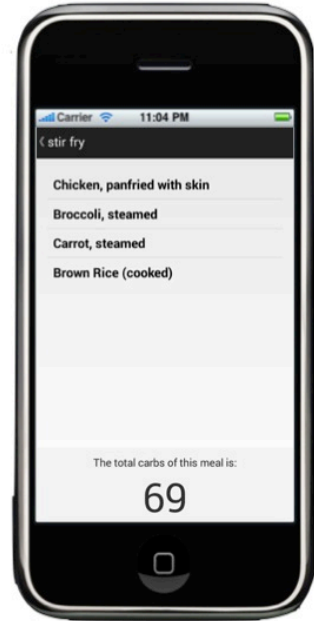
A: Searchable Food List



B: Carbohydrate Content of Selected Food and Portion Size



C: Carb Content of Entered Recipe/Meal



FOOD INSULIN DEMAND (FID) COUNTING

FOOD REFERENCE GUIDE FOR PEOPLE WITH
TYPE 1 DIABETES



Healthy Eating

Healthy eating is an important part of managing your diabetes and your overall health. A healthy, balanced diet should include a wide range of foods from all the different food groups.



Australian Guide to Healthy Eating

Enjoy a wide variety of nutritious foods from these five food groups every day.
Drink plenty of water.



Different people need different amounts of food depending on their energy and nutritional needs.

Talk to an Accredited Practising Dietitian to help you work out a healthy, balanced diet that's right for you.

Insulin & Food

The foods you eat are broken down by the body and are absorbed into the blood stream. Insulin acts like a key, opening the 'door' to the cells of the body so the nutrients can get into the cells for energy and helps lower blood glucose levels.



Food

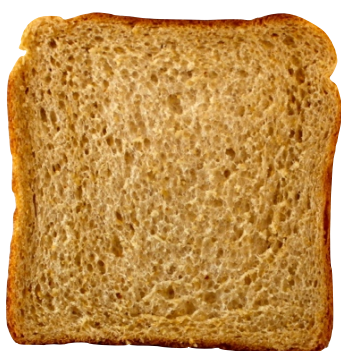
Foods provide the nutrients that the cells of the body need to function properly



Insulin

Insulin acts like a key to open the 'door' to the cells to get the nutrients inside

Different foods require a different amount of insulin to metabolise them. This amount of insulin depends on a number of factors including the amount of carbohydrate, protein and fat in the food and how it is digested. We can 'count' the food insulin demand (FID) of each food and use this to determine how much insulin is needed for the food or meal.



Food Insulin Index

The Food Insulin Index ranks foods according to their relative insulin demand, in other words, how much insulin is needed to metabolise a food compared to other foods. Knowing the insulin demand of the foods you are eating can help you predict how much insulin you need to keep your blood glucose levels in a healthy range.

The **Food Insulin Index (FII)** tells us how much insulin is needed for each food or meal eaten. This is a fixed number and doesn't change depending on how much you eat. For example, the FII of an apple is 43, no matter how much you eat.



Apple
FII: 43



White Bread, 1 Slice
FII: 73
FID: 26

The **Food Insulin Demand (FID)** takes into account the FII and how much you are going to eat. We need to multiply the FII of the food by the number of kJ in your portion size. For example: 1 slice of white bread has a FII of 73 and contains 310kJ, so the FID is 26

Using the FII

The amount of insulin you need is determined using your own insulin: FID ratio. For example, 1 unit of insulin: 16 FID points. This means that for every 16 FID points you eat, you need 1 unit of insulin. Your insulin: FID ratio is based on your insulin sensitivity.

The total amount of insulin you need can be calculated by adding together the FID of each of the foods you are about to eat. This booklet provides the FID for many commonly eaten foods.

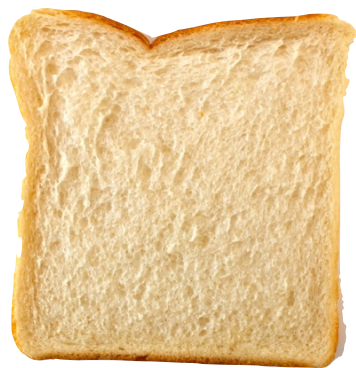


The insulin pumps currently available on the market do not allow for an 'insulin: FID ratio' to be programmed, however you can use the 'insulin: carbohydrate ratio' function the same way. To bolus your mealtime insulin dose, enter the FID for the food or meal you are eating into your insulin pump bolus wizard where you would normally enter the carbohydrate value.

Use your insulin: carbohydrate ratio and enter the FID of your food or meal instead of the carbohydrate

The Foods

Breads



White Bread

1 Slice (35g)

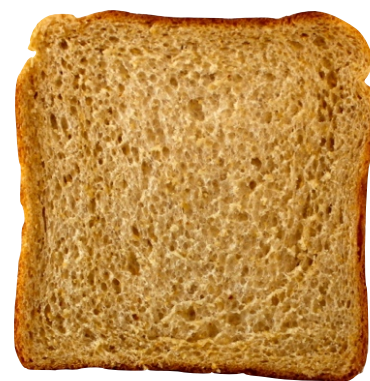
FID: 26



Grain Bread

1 Slice (28g)

FID: 11



Wholemeal Bread

1 Slice (40g)

FID: 28



Soy & Linseed Bread

1 Slice (41g)

FID: 22



Tortilla

1 Tortilla (40g)

FID: 14

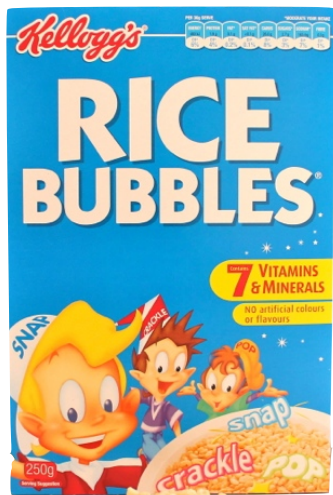


Croissant

1 Medium (50g)

FID: 44

Cereals



Rice Bubbles

30g (1 Cup)

FID: 45



Corn Flakes

30g (1 Cup)

FID: 26



Special K

40g (1 Cup)

FID: 30



Sultana Bran

60g (1 Cup)

FID: 56



Sustain

60g (1 Cup)

FID: 50

Cereals



Cheerios
30g (1 Cup)
FID:29



All-Bran Original
60g (1 Cup)
FID: 19



All-Bran Wheat Flakes
40g (1 Cup)
FID: 29



Honey Weets
30g (1 Cup)
FID: 23



Porridge (Oats Cooked with Water)
1/3 Cup Raw Oats (30g)/1 Cup Cooked
FID: 15

Rice & Pasta



Spiral Pasta
1 Cup Cooked
FID: 22



Wholemeal Pasta
1 Cup Cooked
FID: 20



Brown Rice
1 Cup Cooked
FID: 49



White Rice
1 Cup Cooked
FID: 46

Rice and pasta contain fibre and other nutrients and are generally low in kilojoules. Beware of the sauces you add to these meals as they can add more kilojoules than you imagined.

Fruit



Apple
1 Medium (150g)
FID: 14



Orange
1 Medium (230g)
FID: 11



Banana
1 Medium (170g)
FID: 23



Honeydew Melon
1 Slice (100g)
FID: 7



Sultanas
40g (1/4 Cup)
FID: 17



Grapes
12 Grapes (120g)
FID: 18

Fruit



Peaches Canned in Syrup

1 Cup (250g)

FID: 47



Peaches Canned in Juice

1 Cup (250g)

FID: 28

The Australian Guide to Healthy Eating recommends eating at least 2 serves of fruit each day. Try incorporating a variety of fruits as a healthy snack.

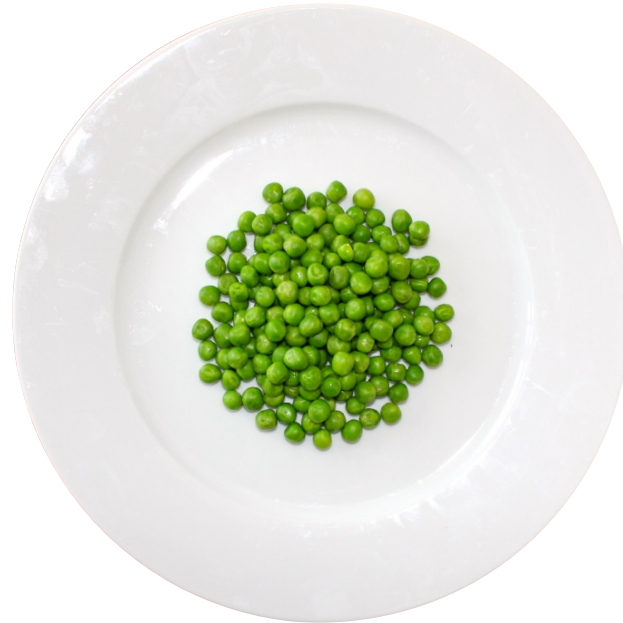
Free Vegetables



Carrots

1 Medium (1/2 Cup, 78g raw)

FID: 6



Peas

1/2 Cup (80g raw)

FID: 9



Broccoli

1/2 Cup (55g raw)

FID: 2



Cauliflower

1/2 Cup (80g raw)

FID: 5

Other Vegetables



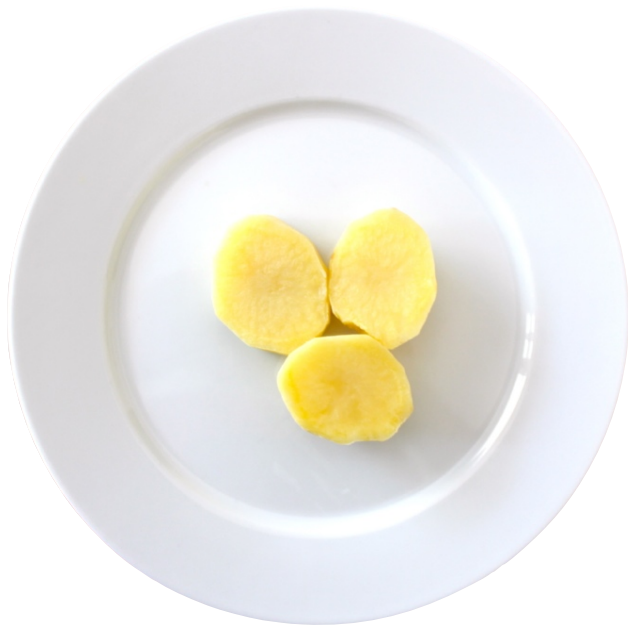
Coleslaw
1/2 Cup (100g)
FID: 8



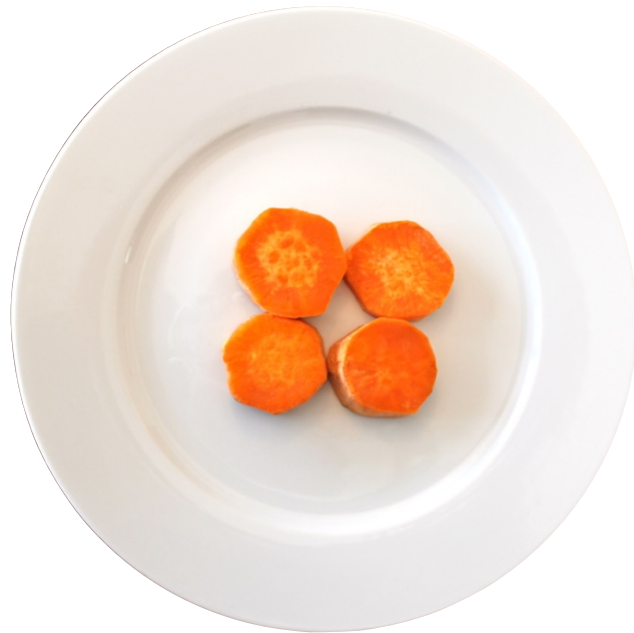
Avocado
1/4 Avocado (50g)
FID: 2

Vegetables low in kilojoules are often referred to as 'free vegetables'. These vegetables are often high in fibre and other nutrients, so they are great for filling up on while still watching your waistline.

Starchy Vegetables



Boiled Potato
1 Medium (150g)
FID: 36



Boiled Sweet Potato
1 Small (120g)
FID: 37



Roast Pumpkin
100g
FID: 15



Corn
1/2 Cup (80g)
FID: 14

Dairy Products



Skim Milk
250mL (1 Cup)
FID: 23



Low Fat Milk
250mL (1 Cup)
FID: 16



Full Cream Milk
250mL (1 Cup)
FID: 17



Low Fat Fruit Yoghurt
175g Tub
FID: 57



Vanilla Ice-Cream
1 Scoop (50g)
FID: 27



Low Fat Vanilla Ice-Cream
1 Scoop (50g)
FID: 19



Fruit Frozen Yoghurt
1 Scoop (50g)
FID: 18

Dairy Products



Cheddar Cheese

1 Slice (25g)

FID: 14



Low Fat Cheddar Cheese

1 Slice (21g)

FID: 4



Low Fat Processed Cheese

1 Slice (20.5g)

FID: 6



Cream Cheese

1 Tablespoon (20g)

FID: 5



Reduced Fat Cottage Cheese

1/2 Cup (120g)

FID: 21



Low Fat Cottage Cheese

1/2 Cup (120g)

FID: 24

Meats & Chicken



Beef Steak

150g Raw, 130g Cooked

FID: 30



Panfried Chicken

150g Raw, 130g Cooked

FID: 26



Roast Chicken

130g Cooked

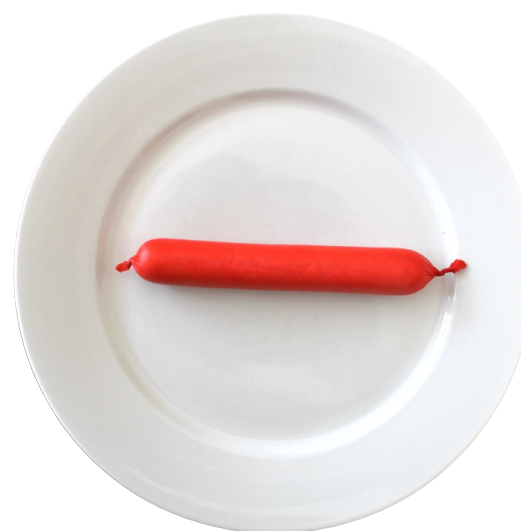
FID: 20



Short-Cut Bacon

2 Rashers (72g)

FID: 6

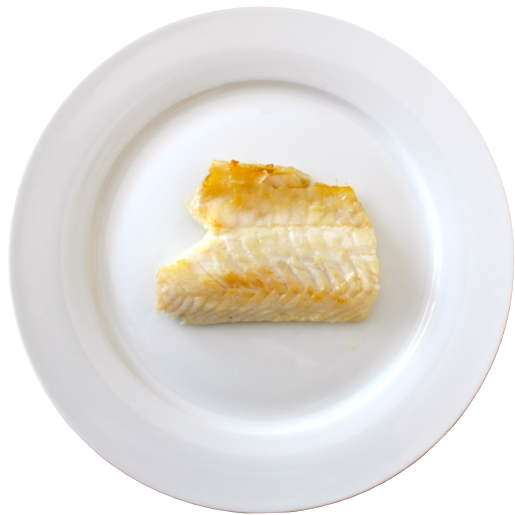


Frankfurter (Hot Dog)

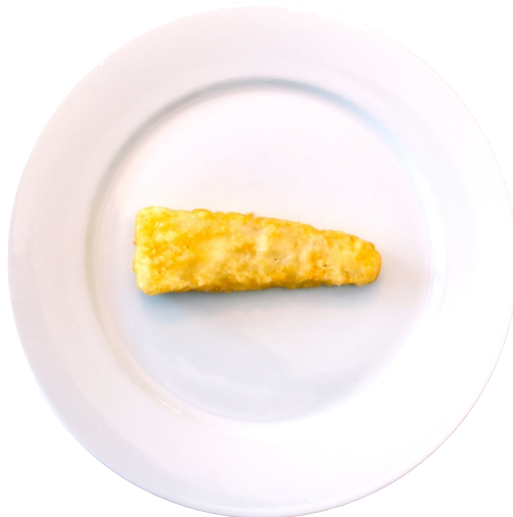
1 Thin (70g)

FID: 12

Seafood



White Fish Fillet
150g Raw, 130g Cooked
FID: 17



Battered Fish Fillet
1 Fillet (70g)
FID: 34



Prawns
7 Shelled Prawns (50g)
FID: 4



Tuna in Olive Oil
95g Tin Drained (80g)
FID: 9



Tuna in Springwater
95g Tin Drained (80g)
FID: 9

Nuts, Eggs & Meat Alt.



Poached Eggs
2 Large Eggs
FID: 14



Tofu
100g
FID: 9



Baked Beans
1/2 Cup (130g)
FID: 33



Salted Peanuts
1/3 Cup (50g)
FID: 12



Walnuts
1/4 Cup (30g)
FID: 4

Meals & Convenience Foods



Cheese Pizza

1 Slice (71g)

FID: 37



Beef Lasagne

400g

FID: 83



Beef Taco

100g

FID: 17



French Fries

100g

FID: 58



McDonald's Fries

1 Small Serve (72g)

FID: 54

Chips & Crackers



Popcorn
1 Cup (25g)
FID: 19



Water Crackers
6 Crackers (18g)
FII: 20



Jatz Crackers
6 Crackers
FID: 22



Corn Chips
11 Chips (27g)
FID: 26



Potato Chips
15 Chips (27g)
FID: 28



97% Fat-Free Pretzels
16 Pretzels (30g)
FID: 35

Confectionary



Mars Bar
1 Regular Bar (53g)
FID: 87



Snickers Bar
1 Regular Bar (60g)
FID: 44



Jellybeans
10 Small Jellybeans
FID: 33



Sherbet
1 Sachet (12.5g)
FID: 4



Milk Chocolate
6 Squares (30g)
FID: 23

Baked Goods



**Chocolate Chip
Cookies**
2 Cookies (25g)
FID: 33



**Reduced Fat
Blueberry Muffin**
1 Large Muffin (170g)
FID: 116



Cinnamon Donut
1 Donut (70g)
FID: 58



Muesli Bar
1 Bar (31g)
FID: 17



Pancake
1 Pancake (100g)
FID: 83



**Chocolate Brownie
with Frosting**
1 Brownie (50g)
FID: 47



Apple Pie
1 Slice (100g)
FID: 55

Sauces & Spreads



Butter
1 Teaspoon (5g)
FID: 0



Peanut Butter
1 Teaspoon (15g)
FID: 4



Raspberry Jam
1 Teaspoon (15g)
FID: 11



Sugar
1 Teaspoon (5g)
FID: 5



Olive Oil
1 Tablespoon (20mL)
FID: 2



Tomato Pasta Sauce
1/2 Cup (125g)
FID: 12

Beverages



Coca-Cola
1 Can (375mL)
FID: 29



Fruit Juice Drink
200mL
FID: 19



Carrot Juice
250mL (1 Cup)
FID: 14



Orange Juice
250mL
FID: 23



Apple Juice
250mL
FID: 21



Ice Tea
1 Bottle (500mL)
FID: 57

Frequently Asked Questions

How is the insulin demand of a food determined?

The insulin demand of a food is tested in people with diabetes. This provides a measure of how much insulin the pancreas would normally produce in response to particular foods. The average response from at least 10 people gives us the Food Insulin Index (FII) for the food. The FID can then be calculated from the FII based on the number of kilojoules in the serve size.

Can the FID be calculated from the food label?

No, the Food Insulin Index (FII) must be tested in healthy people without diabetes. The body is a very complex system and there are many factors contributing to the overall insulin demand of the food. The total amount of carbohydrate, protein and fat plays a role but it also depends on the types of carbohydrate, protein and fat, how the nutrients interact in the food and in the body, how the food is prepared/cooked and how the body digests it. There may also be factors we don't know much about yet. By measuring the insulin demand in people we can capture all these factors into one number.

Why are there only a limited number of foods in this booklet?

Each food needs to be tested by at least 10 people to find the Food Insulin Index (FII) value, so it takes time to add new foods to the list. So far more than 130 commonly eaten foods have been tested and we are constantly testing more foods.

I can't find a food in the booklet. How do I work out the FID?

If there is a similar food in the booklet, use the FID of this food as a guide to the FID of your food. For example; the FID of a pear will be similar to an apple and the FID of mince will be similar to beef steak. If the food is made from a few easily identified ingredients, such as sushi, you can add the FID of the ingredients together to find the FID. For example a sushi roll = rice + chicken or fish + avocado + cucumber). We have provided space at the back of this book to record the FID for any foods or meals you've calculated.

Frequently Asked Questions

How do I work out the FID for a meal or recipe?

The FID of a meal can be worked out by working out the FID of the ingredients in the meal. Make sure you adjust the FID to match the amount added to the meal. The total FID of the meal is calculated by adding together the FID values of each ingredient.

If you are cooking for multiple people or will get multiple meals from the recipe, you may find it easier to work out the FID for the whole meal and then divide by the number of serves in the recipe. There is an example of how to do this in your workshop manual.

I want to eat a different serve size than the one listed in the booklet. How do I adjust the FID?

The listed FID is just an example serve size. You may choose to eat more or less than this. To double the serve size, double the FID and to halve the serve size, halve the FID. The 'FID Counter' iPhone app can be used to do this calculation for you and also lists many other ways of measuring your portion size for you to choose from.

There aren't any FID values for salads, berries or diet products (diet softdrinks, diet cordials). How do I work out the FID for these foods?

These foods are too low in kilojoules to have a FID and so you don't need insulin for these foods. The Food Insulin Index (FII) is tested in 1000kJ portions and these foods don't have enough kilojoules to be tested, so an FID cannot be calculated.

There aren't any FID values listed for alcohol. How do I work out the FID?

The FID values for alcohol has purposely not been added for alcohol as it is not recommended to dose insulin for alcoholic drinks. Alcohol prevents the liver from releasing small amounts of stored glucose to keep your blood glucose levels in a healthy range. Therefore injecting or infusing insulin can further increase the risk of hypoglycaemia associated with alcohol.

Frequently Asked Questions

How do I use the FID to treat my hypoglycaemia?

Hypoglycaemia occurs when blood glucose levels drop below 4mmol/L. To treat hypoglycaemia, blood glucose levels need to be raised back to the normal, healthy level as quickly as possible. Foods containing carbohydrates that are quickly digested are the best way to raise your blood glucose levels. This may include glucose jellybeans, glucose tablets, fruit juice or softdrink (not diet varieties). The FID is useful for determining how much insulin you need to foods but you should use carbohydrates to elevate blood glucose levels quickly.

Any other questions?

Contact Kirstie by email at Kirstine.Bell@sydney.edu.au or by phone on 0400 167 043.

CARBOHYDRATE COUNTING

FOOD REFERENCE GUIDE FOR PEOPLE WITH
TYPE 1 DIABETES



Healthy Eating

Healthy eating is an important part of managing your diabetes and your overall health. A healthy, balanced diet should include a wide range of foods from all the different food groups.



Australian Guide to Healthy Eating

Enjoy a wide variety of nutritious foods from these five food groups every day.
Drink plenty of water.



Use small amounts



Only sometimes and in small amounts



Different people need different amounts of food depending on their energy and nutritional needs.

Talk to an Accredited Practising Dietitian to help you work out a healthy, balanced diet that's right for you.

Insulin & Carbohydrate

Foods containing carbohydrate are digested into a sugar called 'glucose' and are absorbed into your blood stream, raising blood glucose levels. Insulin acts like a key, 'opening the door' to the cells of the body so the glucose can get into the cells for energy and lowering blood glucose levels.



Carbohydrates

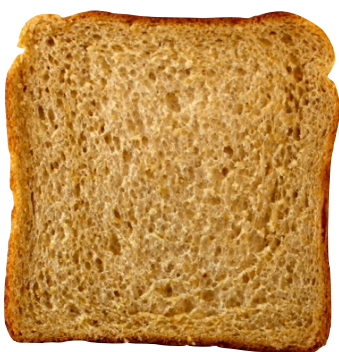
Carbohydrates break down into glucose, raising blood glucose levels.



Insulin

Insulin acts like a key to 'open the door' to the cells so the glucose can get inside, lowering blood glucose levels.

The amount of insulin you need is proportional to the amount of carbohydrate you eat. Therefore we can 'count' the grams of carbohydrate you eat and use this to determine how much insulin you need for that food or meal.



Carbohydrate Counting

Knowing how much carbohydrate is in the foods you eat can help you predict how much insulin you need to keep your blood glucose levels in a healthy range.

Carbohydrate foods can be ‘counted’ by adding together the grams of carbohydrate in each food. For example, 1 apple contains 15g of carbohydrate.



Apple
15g Carbohydrate



White Bread
1 Slice = 35g
Carb: 16g

Remember, the grams of carbohydrate is different to the weight of the food. For example, a slice of white bread weighs 35g but contains 16g of carbohydrate.

Using Carb Counting

The amount of insulin you need is determined using your own insulin: carbohydrate ratio. E.g. 1 unit of insulin: 10g of carbohydrate. This means that for every 10g of carbohydrate you eat, you need 1 unit of insulin. This ratio is prescribed for you based on your insulin sensitivity.

The total amount of insulin you need can be calculated by adding together the amount of carbohydrate (grams) in each of the foods you are about to eat. This booklet provides the amount of carbohydrate in common foods.

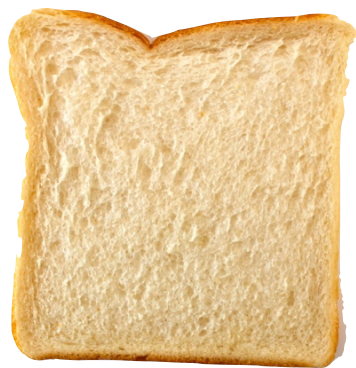


Insulin pumps allow you to pre-program your insulin: carbohydrate ratio, so that you can enter the amount of carbohydrate and the insulin pump will determine the amount of insulin you need.

Use your insulin: carbohydrate ratio and enter the grams of carbohydrate you eat into your bolus wizard

Foods

Breads



White Bread

1 Slice (35g)

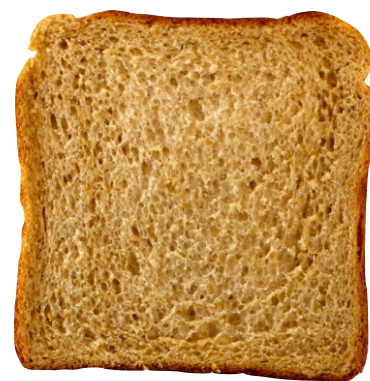
Carb: 16g



Grain Bread

1 Slice (28g)

Carb: 10g



Wholemeal Bread

1 Slice (40g)

Carb: 18g



Soy & Linseed Bread

1 Slice (41g)

Carb: 12g



Tortilla

1 Tortilla (40g)

Carb: 18g

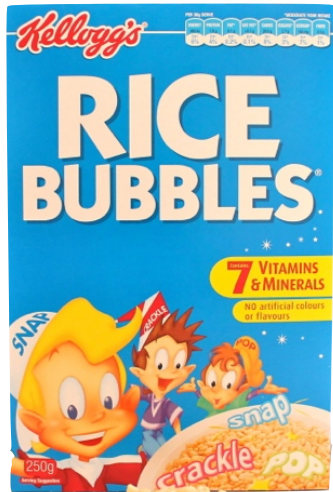


Croissant

1 Medium (50g)

Carb: 22g

Cereals



Rice Bubbles
30g (1 Cup)
Carb: 26g



Corn Flakes
30g (1 Cup)
Carb: 25g



Special K
40g (1 Cup)
Carb: 26g



Sultana Bran
60g (1 Cup)
Carb: 40g



Sustain
60g (1 Cup)
Carb: 41g

Cereals



Cheerios
30g (1 Cup)
Carb: 20g



All-Bran Original
60g (1 Cup)
Carb: 35g



All-Bran Wheat Flakes
40g (1 Cup)
Carb: 25g



Honey Weets
30g (1 Cup)
Carb: 23g



Porridge (Oats Cooked with Water)
1/3 Cup Raw Oats (30g)/1 Cup Cooked
Carb: 19g

Rice & Pasta



Spiral Pasta
1 Cup Cooked
Carb: 36g



Wholemeal Pasta
1 Cup Cooked
Carb: 33g



Brown Rice
1 Cup Cooked
Carb: 58g



White Rice
1 Cup
Carb: 45g

Rice and pasta contain fibre and other nutrients and are generally low in kilojoules. Beware of the sauces you add to these meals as they can add more kilojoules than you imagined.

Fruit



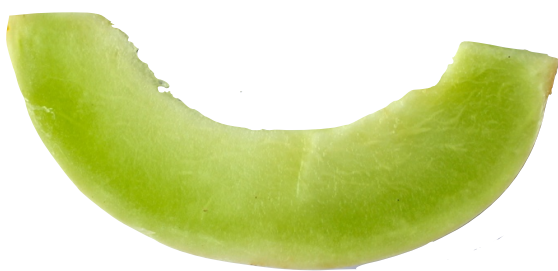
Apple
1 Medium (150g)
Carb: 18g



Orange
1 Medium (230g)
Carb: 13g



Banana
1 Medium (170g)
Carb: 22g



Honeydew Melon
1 Slice (100g)
Carb: 3g



Sultanas
40g (1/4 Cup)
Carb: 31g



Grapes
12 Grapes (120g)
Carb: 17g

Fruit



Peaches Canned in Syrup
1 Cup (250g)
Carb: 40g



Peaches Canned in Juice
1 Cup (250g)
Carb: 28g

The Australian Guide to Healthy Eating recommends eating at least 2 serves of fruit each day. Try incorporating a variety of fruits as a healthy snack.

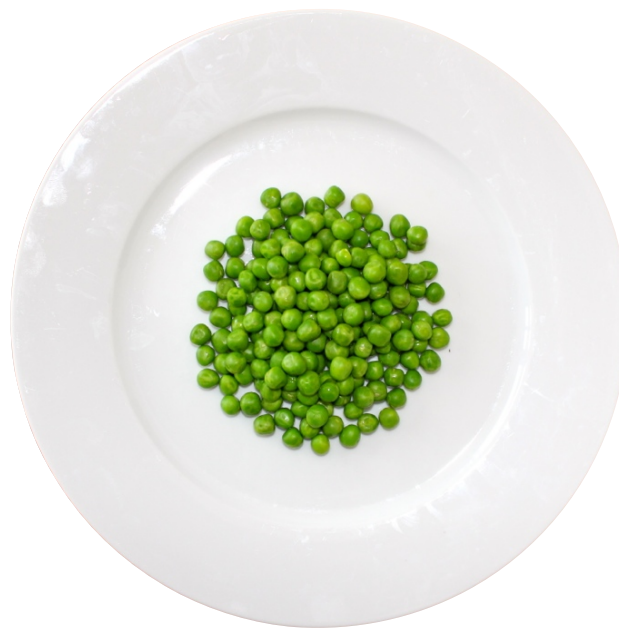
Free Vegetables



Carrots

1 Medium (1/2 Cup, 78g raw)

Carb: 5g



Peas

1/2 Cup (80g raw)

Carb: 5g



Broccoli

1/2 Cup (55g raw)

Carb: 1g



Cauliflower

1/2 Cup (80g raw)

Carb: 2g

Other Vegetables



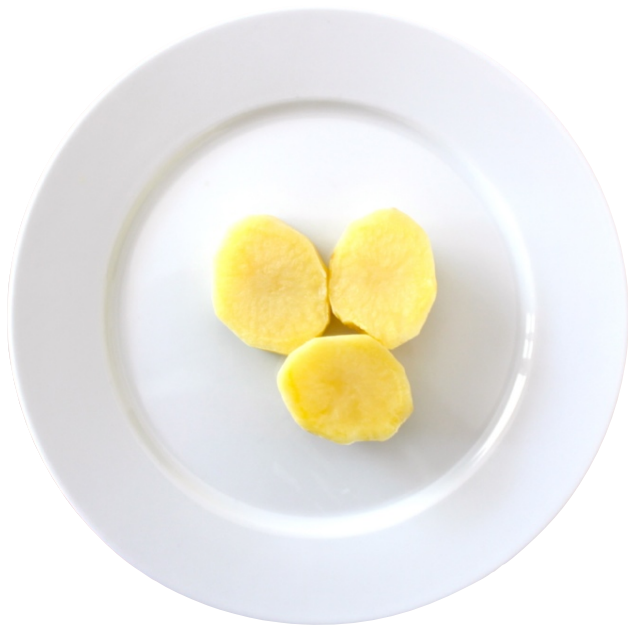
Coleslaw
1/2 Cup (100g)
Carb: 13g



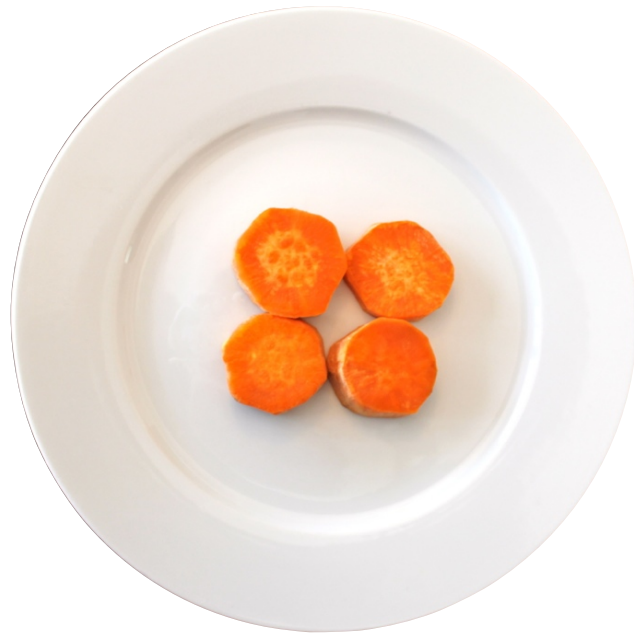
Avocado
1/4 Avocado (50g)
Carb: 0g

Vegetables low in kilojoules are often referred to as ‘free vegetables’. These vegetables are often high in fibre and other nutrients, so they are great for filling up on while still watching your waistline.

Starchy Vegetables



Boiled Potato
1 Medium (150g)
Carb: 20g



Boiled Sweet Potato
1 Small (120g)
Carb: 18g



Roast Pumpkin
100g
Carb: 7g



Corn
1/2 Cup (80g)
Carb: 15g

Dairy Products



Skim Milk
250mL (1 Cup)
Carb: 13



Low Fat Milk
250mL (1 Cup)
Carb: 13g



Full Cream Milk
250mL (1 Cup)
Carb: 13g



Low Fat Fruit Yoghurt
175g Tub
Carb: 26g



Vanilla Ice-Cream
1 Scoop (50g)
Carb: 11g



Low Fat Vanilla Ice-Cream
1 Scoop (50g)
Carb: 12g



Fruit Frozen Yoghurt
1 Scoop (50g)
Carb: 11g

Dairy Products



Cheddar Cheese
1 Slice (25g)
Carb: 0g



Low Fat Cheddar Cheese
1 Slice (21g)
Carb: 0g



Low Fat Processed Cheese
1 Slice (20.5g)
Carb: 2g



Cream Cheese
1 Tablespoon (20g)
Carb: 1g



Reduced Fat Cottage Cheese
1/2 Cup (120g)
Carb: 4g



Low Fat Cottage Cheese
1/2 Cup (120g)
Carb: 7g

Meats & Chicken



Beef Steak

150g Raw, 130g Cooked
Carb: 0g



Panfried Chicken

150g Raw, 130g Cooked
Carb: 0g



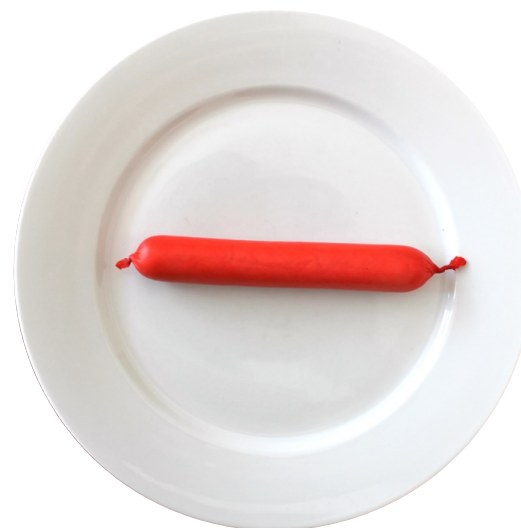
Roast Chicken

130g Cooked
Carb: 0g



Short-Cut Bacon

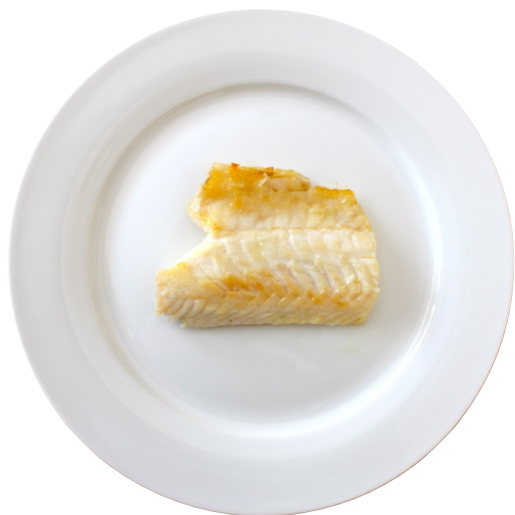
2 Rashers (72g)
Carb: 1g



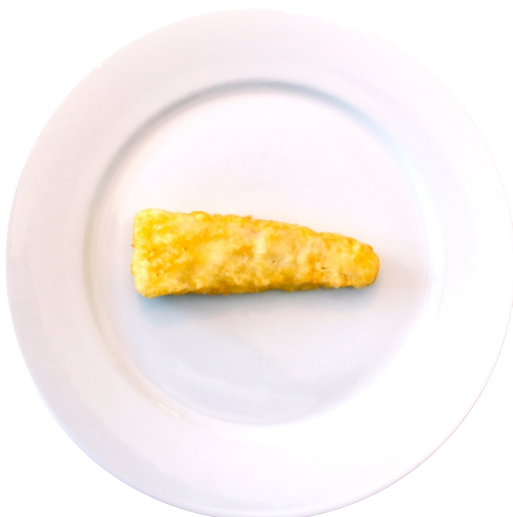
Frankfurter (Hot Dog)

1 Thin (70g)
Carb: 2g

Seafood



White Fish Fillet
150g Raw, 130g Cooked
Carb: 0g



Battered Fish Fillet
1 Fillet (70g)
Carb: 9g



Prawns
7 Shelled Prawns (50g)
Carb: 1g



Tuna in Olive Oil
95g Tin Drained (80g)
Carb: 0g



Tuna in Springwater
95g Tin Drained (80g)
Carb: 0g

Nuts, Eggs & Meat Alt.



Poached Eggs
2 Large Eggs
Carb: 1g



Tofu
100g
Carb: 3g



Baked Beans
1/2 Cup (130g)
Carb: 14g



Salted Peanuts
1/3 Cup (50g)
Carb: 4g



Walnuts
1/4 Cup (30g)
Carb: 1g

Meals & Convenience Foods



Cheese Pizza
1 Slice (71g)
Carb: 23g



Beef Lasagne
400g
Carb: 60g



Beef Taco
100g
Carb: 7g



French Fries
100g
Carb: 40g



McDonald's Fries
1 Small Serve (72g)
Carb: 24g

Chips & Crackers



Popcorn
1 Cup (25g)
Carb: 13g



Water Crackers
6 Crackers (18g)
Carb: 13g



Jatz Crackers
6 Crackers
Carb: 17g



Corn Chips
11 Chips (27g)
Carb: 16g



Potato Chips
15 Chips (27g)
Carb: 16g



97% Fat-Free Pretzels
16 Pretzels (30g)
Carb: 21g

Confectionery



Mars Bar

1 Regular Bar (53g)

Carb: 37g



Snickers Bar

1 Regular Bar (60g)

Carb: 35g



Jellybeans

10 Small Jellybeans

Carb: 16g



Sherbet

1 Sachet (12.5g)

Carb: 3g



Milk Chocolate

6 Squares (30g)

Carb: 17g

Baked Goods



**Chocolate Chip
Cookies**
2 Cookies (25g)
Carb: 17g



**Reduced Fat
Blueberry Muffin**
1 Large Muffin (170g)
Carb: 85g



**Chocolate Brownie
with Frosting**
1 Brownie (50g)
Carb: 24g



Muesli Bar
1 Bar (31g)
Carb: 18g



Cinnamon Donut
1 Donut (70g)
Carb: 28g



Apple Pie
1 Slice (100g)
Carb: 38g

Sauces & Spreads



Butter
1 Teaspoon (5g)
Carb: 0g



Peanut Butter
1 Teaspoon (15g)
Carb: 3g



Raspberry Jam
1 Teaspoon (15g)
Carb: 6g



Sugar
1 Teaspoon (5g)
Carb: 5g



Olive Oil
1 Tablespoon (20mL)
Carb: 0g



Tomato Pasta Sauce
1/2 Cup (125g)
Carb: 11g

Beverages



Coca-Cola
1 Can (375mL)
Carb: 39g



Fruit Juice Drink
200mL
Carb: 15g



Carrot Juice
250mL (1 Cup)
Carb: 13g



Orange Juice
250mL
Carb: 21g



Apple Juice
250mL
Carb: 26g



Ice Tea
1 Bottle (500mL)
Carb: 46g



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The FOODII Study

Food Insulin Demand (FID) Counting

Workshop Manual



Facilitator: Kirstie Bell

APD/AN, CDE & PhD Candidate

Email: Kirstine.Bell@sydney.edu.au

Session Overview

Time	Topic/Activity	Page
	Introduction	
	What is FID Counting?	
	How to use FID Counting in Practice	
	Activity: Food Diary	3
	Activity: Estimating Meals & Recipes	4
	Estimating Food Portion Size	6
	Activity: Weigh & Measure Foods	7
	Summary	

Activity 2:
Figuring out Recipes & Meals

The FID of a meal can be calculated by working out the FID of the ingredients in the meal. Make sure you adjust the FID to match the amount added to the meal. The total FID of the meal is calculated by adding together the FID values of each ingredient.

If you are cooking for multiple people or will get multiple meals from the recipe, you may find it easier to work out the FID for the whole meal and then divide by the number of serves in the recipe. There is an example of how to do this in your workshop manual.

Ingredient	Amount	FID
	Total FID <i>(Add up all FID)</i>	
	Number of Serves <i>(How many serves did it make?)</i>	
	FID per Serve <i>(Divide 'Total FID' by 'Number of Serves')</i>	

Example: Chicken Stir-fry

Ingredient	Amount	FID
<i>Rice</i>	<i>4 cups cooked</i>	<i>184 (46 x 4)</i>
<i>Chicken</i>	<i>300g Raw</i>	<i>52 (26 x 2)</i>
<i>Carrot</i>	<i>2 medium</i>	<i>12 (6 x 2)</i>
<i>Broccoli</i>	<i>1 cup</i>	<i>4 (2 x 2)</i>
<i>Cauliflower</i>	<i>1 cup</i>	<i>10 (5 x 2)</i>
Total FID <i>(Add up all FID)</i>		262
Number of Serves <i>(How many serves did it make?)</i>		4
FID per Serve <i>(Divide 'Total FID' by 'Number of Serves')</i>		65.5

So for 1 serving of the Chicken Stir-fry, the FID is 65.5



Estimating Portion Sizes

A key element of FID counting is being able to accurately estimate your portion size. The chart below gives some examples of what some common portion size measures look like compared to household objects.

1 Cup =



Baseball

$\frac{3}{4}$ Cup =



Tennis Ball

$\frac{1}{2}$ Cup =



Computer Mouse

$\frac{1}{4}$ Cup =



Egg

85g =



Deck of Cards

2 Teaspoons =



Ping Pong Ball

Adapted from www.snacksense.com

Activity 3:
Weighing it Up

Serve yourself the amount of the food you would normally eat into the bowl provided. Use the sheet below to record your estimations.

Part 1:

Estimate the weight of the food – in grams or measuring cups.

Based on your estimated portion size, estimate the FID of the serve.

Part 2:

Weigh your serve or pour into a measuring cup – how close were your estimations?

Using the reference booklets provided, estimate the actual FID of the serve.

Food	Estimated Weight	Actual Weight	Estimated FID	Actual FID
Rice				
Pasta				
Juice				
Apple				
Cereal				
Meat				



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The FOODII Study

Carbohydrate Counting

Workshop Manual



Facilitator: Kirstie Bell

APD/AN, CDE & PhD Candidate

Email: Kirstine.Bell@sydney.edu.au

Session Overview

Time	Topic/Activity	Page
	Introduction	
	What is Carb Counting?	
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	Activity: Food Diary	3
	Activity: Estimating Meals & Recipes	4
	Estimating Food Portion Size	6
	Activity: Weigh & Measure Foods	7
	Summary	

Activity 1:
Carbohydrate Counting – Food Diary

Complete the following tables by filling in the foods and the amounts you have eaten today (or what you would normally eat in a day) and then estimating the carbohydrate content using your reference booklets.

Food	Amount	Carb
<i>Apple</i>	<i>1 medium</i>	<i>18g</i>

Activity 2:
Figuring out Recipes & Meals

The carbohydrate content of a meal can be calculated by working out the carbohydrate content of the ingredients in the meal. Make sure you adjust the carbs to match the amount added to the meal. The total carb of the meal is calculated by adding together the grams of carb in each ingredient.

If you are cooking for multiple people or will get multiple meals from the recipe, you may find it easier to work out the grams of carbohydrate in the whole meal and then divide by the number of serves in the recipe. There is an example of how to do this in your workshop manual.

Ingredient	Amount	Carb (g)
	Total Carb <i>(Add up all grams of carb)</i>	
	Number of Serves <i>(How many serves did it make?)</i>	
	Carb per Serve <i>(Divide 'Total Carb' by 'Number of Serves')</i>	

Example: *Chicken Stir-fry*

Ingredient	Amount	Carbs (g)
<i>Rice</i>	<i>4 cups cooked</i>	<i>180 (45g x 4)</i>
<i>Chicken</i>	<i>300g Raw</i>	<i>0</i>
<i>Carrot</i>	<i>2 medium</i>	<i>10 (5g x 2)</i>
<i>Broccoli</i>	<i>1 cup</i>	<i>2 (1g x 2)</i>
<i>Cauliflower</i>	<i>1 cup</i>	<i>4 (2g x 2)</i>
Total Carb <i>(Add up all grams of carb)</i>		196
Number of Serves <i>(How many serves did it make?)</i>		4
Grams of Carb per Serve <i>(Divide 'Total Carb' by 'Number of Serves')</i>		49

So 1 serving of the Chicken Stir-fry, contains 49g of carbohydrate



Estimating Portion Sizes

A key element of carbohydrate counting is being able to accurately estimate your portion size. The chart below gives some examples of what some common portion size measures look like compared to household objects.

1 Cup =



Baseball

3/4 Cup =



Tennis Ball

1/2 Cup =



**Computer
Mouse**

1/4 Cup =



Egg

85g =



Deck of Cards

2 Teaspoons =



Ping Pong Ball

Activity 3:
Weighing it Up

Serve yourself the amount of the food you would normally eat into the bowl provided. Use the sheet below to record your estimations.

Part 1:

Estimate the weight of the food – in grams and in measuring cups.

Weigh your serve – how close were your estimations?

Pour into a measuring cup – how close were your estimations?


Part 2:

Estimate the amount of carbohydrate in the serve.

Using the reference booklets provided, estimate the actual amount of carbohydrate in the serve.

Food	Estimated Weight	Actual Weight	Estimated Carb	Actual Carb
Rice				
Pasta				
Juice				
Apple				
Cereal				
Potato				

Excerpts from the pocket-sized guides




The FOODII Study

**FID Counter:
Pocket-Sized
Guide**

Dairy Products

Food	Serve Size	FID
Skim Milk	250mL	23
Full Cream Milk	250mL	17
Low Fat Fruit Yoghurt	175g Tub	57
Vanilla Ice-Cream	1 Scoop (50g)	27
Low Fat Vanilla Ice-Cream	1 Scoop (50g)	19
Fruit Frozen Yoghurt	1 Scoop (50g)	18
Cheddar Cheese	1 Slice (25g)	14
Low Fat Cheddar Cheese	1 Slice (21g)	4
Low Fat Processed Cheese	1 Slice (20.5g)	6
Cream Cheese	1 Tablespoon (20g)	5
Reduced Fat Cottage Cheese	½ Cup (120g)	21
Low Fat Cottage Cheese	½ Cup (120g)	24

9



The FOODII Study

**Carb Counter:
Pocket-Sized
Guide**

Dairy Products

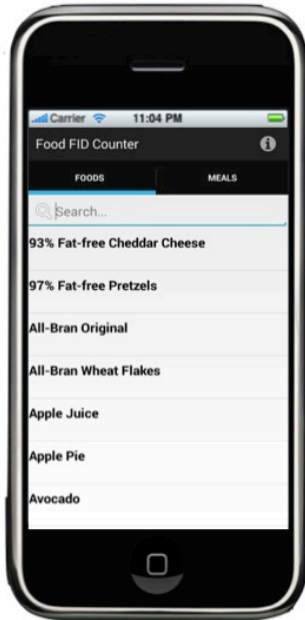
Food	Serve Size	Carbs (g)
Skim Milk	250mL	13
Full Cream Milk	250mL	13
Low Fat Fruit Yoghurt	175g Tub	26
Vanilla Ice-Cream	1 Scoop (50g)	11
Low Fat Vanilla Ice-Cream	1 Scoop (50g)	12
Fruit Frozen Yoghurt	1 Scoop (50g)	11
Cheddar Cheese	1 Slice (25g)	0
Low Fat Cheddar Cheese	1 Slice (21g)	0
Low Fat Processed Cheese	1 Slice (20.5g)	2
Cream Cheese	1 Tablespoon (20g)	1
Reduced Fat Cottage Cheese	½ Cup (120g)	4
Low Fat Cottage Cheese	½ Cup (120g)	7

9

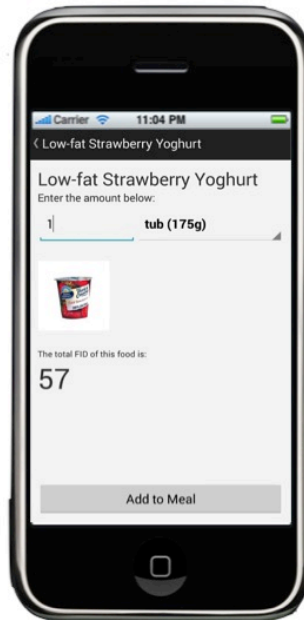
Screenshots of the Smartphone Apps

FID Counter

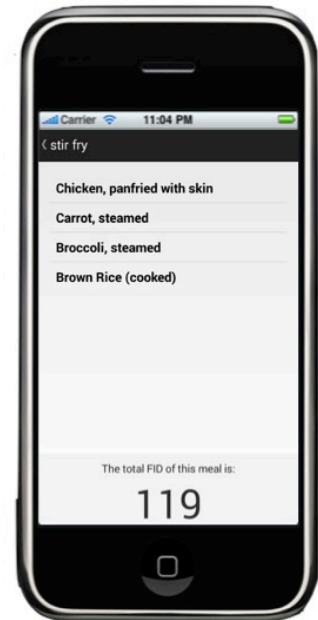
A: Searchable Food List



B: FID of Selected Food and Portion Size

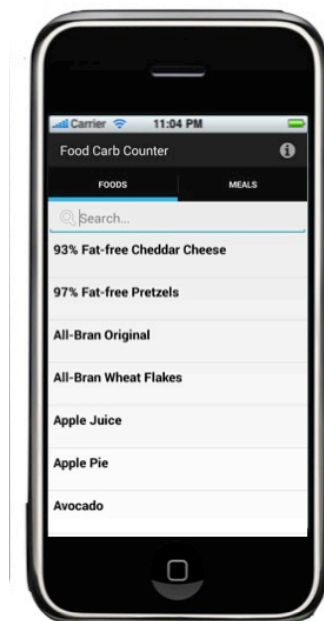


C: FID of Entered Recipe/Meal

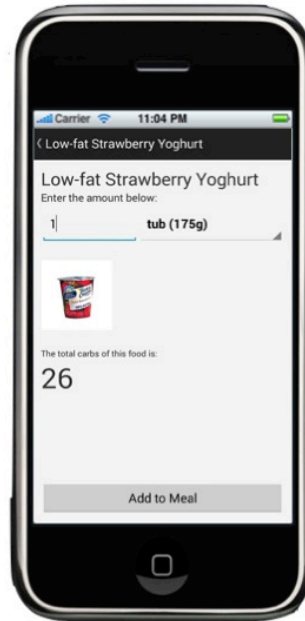


Carb Counter

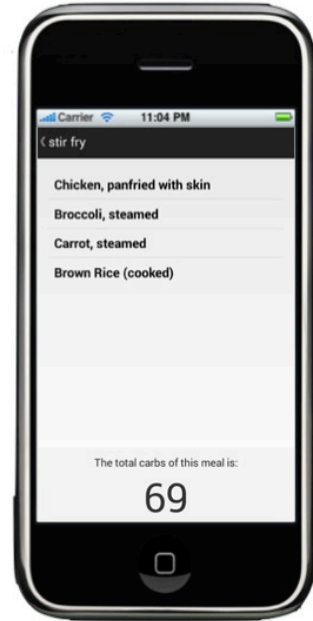
A: Searchable Food List



B: Carbohydrate Content of Selected Food and Portion Size



C: Carb Content of Entered Recipe/Meal



CHAPTER 6

Effect of diets of varying Food Insulin Index
on day-long glucose and insulin profiles in
adults with type 2 diabetes

6.1 Abstract

Introduction: Chronic hyperinsulinaemia in type 2 diabetes may exacerbate insulin resistance and β -cell failure. The Food Insulin Index (FII) is a novel algorithm for ranking foods based on their insulin responses ('demand') in healthy adults relative to an isoenergetic reference food. Our aim was to compare day-long plasma glucose and insulin responses of patients with type 2 diabetes to two diets of varying insulin demand.

Methods: On two separate mornings, 10 adults with type 2 diabetes consumed either a high FII or low FII diet in random order. Diets consisted of three consecutive meals (breakfast, morning tea and lunch), matched for macronutrients, fibre and GI but with two-fold differences in insulin demand as predicted by the FII of the component foods. Postprandial glycaemia and insulinaemia were measured through capillary blood samples at regular intervals over 8 hours.

Results: Compared with the high FII diet, mean postprandial insulin response over 8 h was 41% lower on the low FII diet (iAUC_{insulin} 18,740 \pm 3,100 pmol/L vs. 11,000 \pm 1,810 pmol/L, $p = 0.018$). As predicted, there were no differences in glycaemic responses between the two diets over the same timeframe (iAUC_{glucose} 840 \pm 230 mmol/L vs. 880 \pm 230 mmol/L, $p = 0.994$).

Conclusion: The novel FII algorithm may be a useful tool for reducing postprandial hyperinsulinaemia in type 2 diabetes, thereby potentially improving insulin resistance and β -cell function.

6.2 Introduction

Type 2 diabetes is characterised by chronic hyperglycaemia resulting from insulin resistance and β -cell failure. Insulin resistance is linked to chronic hyperinsulinaemia, which in turn may induce oxidative stress and exacerbate β -cell failure¹. Dietary patterns which induce excessive insulin secretion have been associated with weight gain² and a higher risk of developing type 2 diabetes³.

Conventional diet therapy for type 2 diabetes is based on improving insulin sensitivity, primarily through weight loss, and reducing postprandial hyperglycaemia by manipulating carbohydrate amount, type and distribution over the day⁴. However, protein and fat have also been shown to be potent modulators of insulin secretion in both healthy subjects and those with type 2 diabetes⁵.

The Food Insulin Index (FII) is a novel algorithm for ranking foods based on their physiological insulin demand relative to an isoenergetic reference food in healthy subjects⁶. In single food studies, it has been shown to be a better predictor of observed insulin responses than the carbohydrate content or glycaemic index (GI)⁷. In the context of realistic mixed meals, the FII also predicted day-long glucose and insulin responses in healthy subjects^{8,9}. But this may not be true of individuals with type 2 diabetes because β -cell function and insulin secretion are compromised. The aim of the present study therefore was to determine whether the FII could predict metabolic responses in adults with type 2 diabetes consuming 3 consecutive meals that had similar macronutrient content but a 2-fold difference in insulin demand as predicted by the FII of the component foods. We hypothesised that despite the same carbohydrate content, fibre and GI, there would still be a 2-fold difference in insulin response calculated as area under the curve.

6.3 Methods

6.3.1 Study design

A group of 11 adults with type 2 diabetes were recruited. Volunteers were included if they were well controlled by diet or oral hypoglycaemic agents. They were ineligible if they used insulin therapy, had food allergies or intolerances, or were not proficient in English. The study was approved by the University of Sydney Human Research Ethics Committee and participants gave written, informed consent (See **Appendices 19 – 22** for participant forms and questionnaires).

Subjects presented to the metabolic kitchen after an 8-hour overnight fast. On the day before each test session, subjects were instructed to consume a high carbohydrate, low fat dinner (not standardised), avoid alcohol and legumes, and maintain usual physical activity levels. On two separate test days, subjects consumed a high FII and a low FII diet in random order, consisting of three consecutive meals over an 8-hour period, ie breakfast, morning tea and lunch given at approximate 08:00, 11:00 and 13:00 h respectively. The two diets were matched for energy, macronutrients, fibre and GI but had a 2-fold difference in predicted insulin demand (**Table 6.1**). Capillary blood samples were taken every 30 – 60 minutes, with a total of 13 blood samples taken per test day. Plasma glucose was analysed using the glucose hexokinase enzymatic assay on a centrifugal analyser (Model Hitachi 912; Hitachi, Tokyo, Japan) and plasma insulin was measured by antibody-coated tube radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA).

6.3.2 Statistical analysis

A sample size was estimated based on previous data suggesting 10 subjects were needed to detect one standard deviation difference in insulin incremental area under the curve (iAUC),

with $p < 0.05$ (unpublished data). Data are presented as means \pm SEM. The incremental area under the curve (iAUC) across the 8 h period for glucose and insulin was calculated according to the trapezoidal rule with fasting level (0 time) as the baseline and the area beneath the fasting level ignored¹⁰. Statistical analysis was performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). Differences were considered significant if $p < 0.05$ and highly significant if $p < 0.001$.

6.4 Results

Of the 11 adults (6 men, 5 women) recruited for the study, 10 completed both test sessions. One subject withdrew from the study due to discomfort from capillary blood sampling. The subjects had a mean age of 63 y (range 44 – 78 y), with a mean BMI of 29.6 ± 2.0 kg/m².

As predicted, there was no significant difference in the plasma glucose iAUC over 8 h to the low and high FII diets (840 ± 230 vs 880 ± 230 mmol/L.min respectively, $p = 0.994$, **Table 6.2**). Similarly, there were no significant differences in the mean blood glucose level across 8 h (9.9 ± 1.3 vs 10.3 ± 1.6 mmol/L.min, $p = 0.485$).

In contrast, the mean postprandial insulin iAUC over 8 h was 41% lower on the low FII diet compared with the high FII diet ($11,000 \pm 1,810$ vs $18,740 \pm 3,100$ pmol/L.min respectively, $p = 0.018$, **Fig 6.1, Table 6.2**). Mean blood insulin concentration was 38% higher on the high vs low FII diet (377 ± 50 pmol/L vs 273 ± 63 mmol/L respectively) but the difference did not reach statistical significance ($p = 0.271$).

Table 6.1: Macronutrient composition, glycaemic index (GI), calculated glycaemic load (GL) and food insulin index (FII).

	Energy (kJ)	AvCHO (g)	Fat (g)	Fibre (g)	Protein (g)	GI (%)	GL (g)	FII (%)
HIGH FII DIET								
Breakfast	2500	76	16	14	36	45	34	65
63g white bread								
302g low fat yoghurt								
104g poached egg								
Morning Tea	1000	33	11	1	3	62	20	73
36g cookie								
30g Mars Bar								
Lunch	2500	76	17	14	35	44	33	62
82g whole-meal bread								
53g cheese								
254g baked beans								
TOTAL	6000	185	44	29	74	48	87	65
LOW FII DIET								
Breakfast	2500	73	19	21	33	33	24	35
55g All-Bran cereal								
275mL full-cream milk								
99g grain bread								
Morning Tea	1000	32	12	3	4	63	19	18
18g walnuts								
36g raisins								
Lunch	2500	79	15	5	35	48	38	31
162g corn								
113g roast chicken								
104g tortilla								
TOTAL	6000	184	46	29	72	44	81	30

Table 6.2: Fasting and day-long glucose and insulin profiles for 10 adults with non-insulin treated type 2 diabetes following a low FII and high FII diet.

Diet	Mean Fasting Blood Glucose Level (mmol/L)	Mean Fasting Insulin Level ($\mu\text{mol/L}$)	Mean Postprandial Blood Glucose Level (mmol/L)	Mean Postprandial Blood Insulin Level ($\mu\text{mol/L}$)	Glucose 180min iAUC (mmol/L*min)	Insulin 180min iAUC (pmol/L*min)
High FII	8.3 \pm 0.9	148 \pm 29	9.9 \pm 1.3	377 \pm 50	840 \pm 230	18,740 \pm 3,100
Low FII	8.9 \pm 1.1	151 \pm 51	10.3 \pm 1.6	273 \pm 63	880 \pm 230	11,000 \pm 1,810
P Value	0.096	0.096	0.485	0.271	0.994	0.018

Data presented as mean \pm SEM

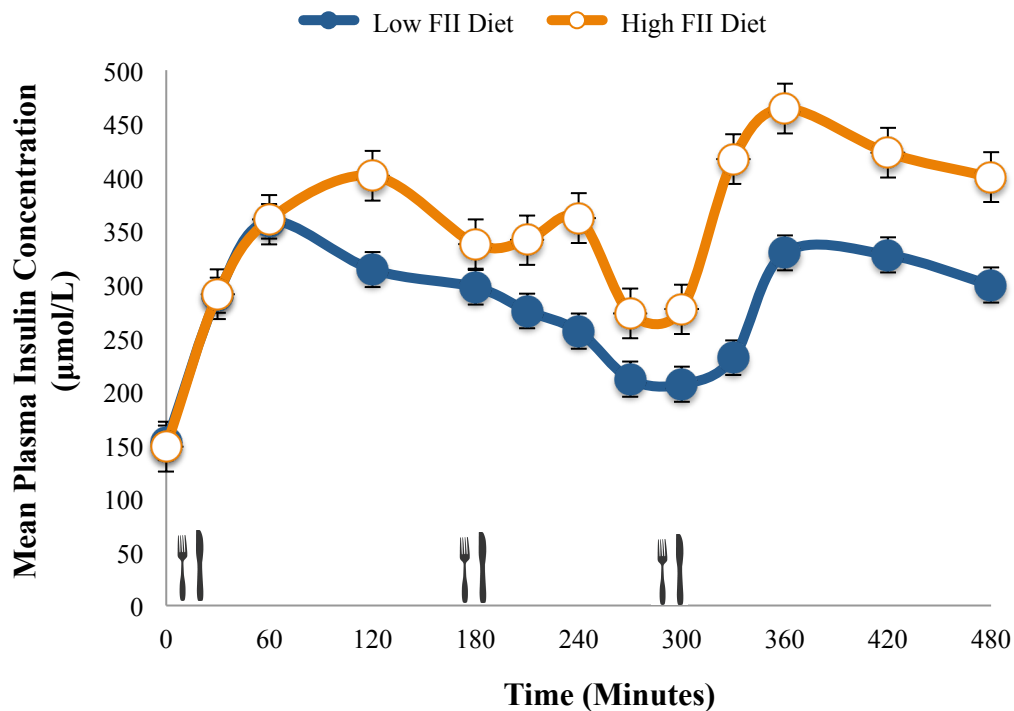


Figure 6.1: High FII vs Low FII Diet over 8 hours on the Mean Plasma Insulin Profile in Adults with Type 2 Diabetes (n=10)

6.5 Discussion

This study provides the first evidence that the novel FII may be applicable to individuals with type 2 diabetes. The results show a 41% reduction in the predicted insulin demand to 3 consecutive meals consumed over the course of 8 h. This occurred despite the fact that glycaemia, macronutrients, fibre and GI were deliberately designed to be similar. The findings suggest that a low FII diet can reduce nutrient-induced hyperinsulinaemia and thereby preserve residual β -cell function in type 2 diabetes.

These results are comparable with those achieved in healthy subjects. A study by Bao et al⁸ showed the low FII diet produced a 53% reduction in mean insulin response (iAUC) over 8 h with no significant differences in the glycaemic response. The small difference in the degree

of reduction of insulinaemic response may be attributable to β -cell dysfunction characteristic of type 2 diabetes.

Carbohydrate, protein and fat have been shown to be potent stimuli for insulin secretion, although varying in their efficacy and mode of action⁵. Therefore the two diets employed in this study were carefully matched for macronutrients, fibre and GI to rule out differences in glycaemic and insulinaemic responses due to macronutrient composition. The two-fold difference in insulin demand was achieved by varying the food sources according to their insulin demand as predicted by the FII. For example, protein-rich foods included in the high FII diet, such as milk, yoghurt, cheese and baked beans, produce an insulin response more than twice that of other protein sources incorporated into the low FII diet, such as roast chicken and walnuts. Although almost all protein sources have been shown to exert an insulinotropic effect, certain protein sources and amino acids, including glutamine, alanine and arginine, have been shown to stimulate β -cell secretion, increasing insulin responses by over 200%^{5, 11, 12}.

As expected, there were no significant differences in the glycaemic responses between the two diets over the same timeframe. Since the two diets were matched for carbohydrate and GI, these factors were not responsible for the observed differences in the glycaemic, and corresponding insulinaemic responses. The effect on insulinaemia could therefore be attributed to fat and protein per se.

This is a physiologically important phenomenon because insulin is required for the metabolism of carbohydrate, protein and fat (and therefore is secreted in response to their ingestion). In order to prevent blood glucose levels from falling as insulin secretion increases, protein also triggers glucagon release¹³. This hormone acts in opposition to insulin, increasing hepatic glucose output and thus maintaining stable blood glucose levels.

This study formed part of a series of studies to document the clinical utility of the novel FII across different groups of the population⁶⁻⁹. The overall aim is to determine whether the use of a FII can produce better clinical outcomes than carbohydrate counting or the use of the GI and GL. It is possible that foods and diets with a lower physiological insulin demand may improve glucose and lipid metabolism in overweight individuals and those with type 2 diabetes, and thereby reduce the risk of complications. For example, Iozzo et al.¹⁴ showed that even a small increase in hyperinsulinaemia in healthy individuals reduced insulin sensitivity, and therefore fostered a vicious cycle of worsening insulin resistance and β -cell dysfunction. Although the FII values are determined by tests in healthy subjects, this study shows the FII is relevant for improving insulinaemic control in people with type 2 diabetes. Due to insulin resistance, the insulin response to protein is ~4-fold greater in people with diabetes than that of healthy subjects¹⁵. Furthermore, ingestion of glucose and protein in healthy subjects has merely an additive effect on insulin secretion, whereas in type 2 diabetes, the serum response is greater than the sum of the individual responses to protein or glucose, indicating there is a synergistic effect on insulin secretion in people with type 2 diabetes¹⁶. Despite these metabolic differences between healthy and type 2 diabetic subjects, the relative difference in insulin demand between foods remains comparable and thus the FII is still applicable to this population.

This study has limitations that should be noted. It was conducted in overweight adults who did not require insulin to manage their diabetes. Therefore, the utility of this dietary strategy will still need to be explored in adults with little residual pancreatic β -cell function. Further research is also warranted to determine if low FII diets can produce clinically meaningful improvements in metabolic control in free-living adults over a longer period of time. The strength of a FII is that it represents a more holistic and practical approach that focuses on whole foods, rather than specific macronutrients within foods. However, it is critical that

further testing of the FII of single foods is continued in order to translate this information into practical food choices. It will always be important to consider not just the overall insulin demand of the diet but also the quantity of food consumed, since even a large portion of a low FII food would produce a significant insulin response.

In summary, a low FII diet produced a significantly lower day-long insulin response compared to a high FII diet, even when matched for macronutrients, fibre and GI. The FII algorithm may therefore be a useful dietary strategy for reducing postprandial hyperinsulinaemia in type 2 diabetes, thereby potentially reducing insulin resistance and preserving β -cell function.

6.6 References

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CHAPTER 7

Discussion and directions
for future research

7.1 Summary of major findings

The achievement of optimal glycaemic control is central to the management of diabetes, yet remains difficult to achieve in day-to-day practice. Given the risk of life-threatening acute and chronic diabetic complications resulting from inadequate glycaemic control, improving the insulin dose algorithm presents a significant clinical issue. This body of research deepens our knowledge of the relationship between foods and normal physiological insulin demand and explores the clinical application of a novel 'insulin index' of foods to diabetes management.

The first chapter of this thesis distilled the current literature on insulin secretion in response to foods and nutrients in healthy individuals, and the role of diet in the management of diabetes.

The concept of a food insulin index (FII) was described in detail, together with its early applications in nutritional epidemiology and type 1 diabetes. At the time of commencement of this candidature, 121 single foods had been tested for their FII¹ and two acute mixed meal studies, one in healthy subjects² and one in type 1 diabetes³, had been undertaken. The challenge was to take the next steps necessary to investigate if the FII was a worthy alternate strategy for estimating insulin dose in the routine management of diabetes.

The broad aims of this research were therefore to apply the FII to adults with diabetes mellitus in a clinical setting and determine the effect of the novel algorithm on acute and chronic glycaemic control compared to carbohydrate counting. We hypothesised that the FII would improve blood glucose control beyond that of carbohydrate counting, without increasing the risk of hypoglycaemia.

The objective of the second chapter of this thesis was to critically explore the scientific evidence for and against carbohydrate counting in order to determine the scientific basis of current clinical practice and provide a benchmark for alternative strategies for mealtime

insulin dosing. We therefore conducted a systematic review and meta-analysis of RCTs of carbohydrate counting in type 1 diabetes according to the PRISMA guidelines. The PRISMA guidelines are based around an evidence-based, 27-item checklist for reporting systematic reviews and meta-analyses to ensure complete and transparent reporting. Surprisingly, we found that current international clinical guidelines for dietary management of type 1 diabetes are based on simple narrative reviews and grading of the available evidence. Only 7 quality RCT could be identified (average quality score 7.6/13), highlighting how little high level evidence exists. This suggests that carbohydrate counting had been integrated into clinical practice long before there was sufficient evidence to support it. The existing studies indicate that carbohydrate counting is not consistently associated with an improvement in glycaemic control, with some studies supporting carbohydrate counting while others suggested less quantitative methods were superior or equally effective. Meta-analyses showed that overall there was no clinically or statistically significant improvement in glycated haemoglobin with carbohydrate counting (-0.35% , $p = 0.01$). The systematic review demonstrated the clinical and practical shortcomings of current therapy and highlighted the need for further research into alternative methods of matching mealtime insulin doses to food intake.

Chapter 3 presented the results of testing 26 new foods for their FII value in healthy subjects and exploratory analysis of the complete database with a total 147 foods. The larger FII database revealed wide variations in the observed insulin responses both within and between food groups. Correlations between the FII and different nutrients indicated that postprandial insulinaemia is not the response to a single nutrient (carbohydrate) but rather the sum total effect of metabolic *interactions* among different nutrients within foods. Although a predictive equation based on knowledge of the GL and protein content could be generated, this

explained only 57% of observed insulin responses in healthy individuals. Because the FII cannot be generated with sufficient precision from the known nutrient content of the food, the conclusion is that the FII of a unknown food is best determined through *in vivo* testing as it cannot be accurately calculated based on the known nutrient content of the food.

In 2011, Bao and colleagues demonstrated that the FII algorithm was superior to carbohydrate counting for estimating the most appropriate insulin dose for two different breakfast meals³. Under carbohydrate counting, insulin would normally be matched to the carbohydrate content of the meal, disregarding the protein and fat content. The FII, however, reveals a significant insulin demand for foods high in protein but containing little to no carbohydrate. As insulin would not typically be administered for protein foods due to fear it would lead to severe hypoglycaemia, it was crucial to determine the safety and efficacy of using the FII to estimate the prandial insulin dose when protein foods were consumed in isolation (a ‘worst case scenario’).

Hence, in the next stage of this research, we specifically chose protein-containing foods where apparent insulin demand was at least 2-fold higher according to the FII as compared to carbohydrate counting. Indeed, two of the test foods (steak and eggs) contained no carbohydrate. It was therefore conceivable that using the FII to estimate insulin dose might result in hypoglycaemia. However, compared with carbohydrate counting, the FII algorithm was able to reduce mean blood glucose levels, without significantly increasing the risk of hypoglycaemia *vis a vis* carbohydrate counting. Because the rate of hypoglycaemia was unusually high in both conditions, further research is warranted.

The protein study described above was also the first to explore the concept of ‘food insulin demand’ as defined by $FID = FII \times \text{kJ per serving}/1000$, a formula for translating the relative FII values into units proportional to the food portion size. The FID allows a mealtime insulin

dose to be calculated based on the FII of the food (or foods) to be consumed and the energy content per serving. This concept has important clinical implications, as it offers a practical approach to implementing the FII. The FII itself has been challenged for being ‘good in theory’ but not feasible in practice as it would be too difficult to use in everyday life. The FID, however, translates the FII into a set of numbers that can be taught to patients in exactly the same way as carbohydrate counting.

Once the safety and efficacy of the FII had been trialled in the research setting, the efficacy of the FII needed to be demonstrated in the normal clinical setting. The research thus far had focused on the *acute* postprandial glycaemic response and it was not known if the use of the FII would translate into clinically meaningful reductions in long-term glycaemic control. Therefore, the fourth study in this thesis was a 12-week RCT comparing the FII with carbohydrate counting for estimating insulin dosage on postprandial glycaemia and HbA1c in adults with type 1 diabetes. As a pilot study, it was the first attempt to teach participants how to use the FII and represented the translation of findings from the research environment to the real-world setting. It was not known if the use of the FII in practice would translate into clinically meaningful reductions in long-term glycaemic control. This study utilised the FID algorithm developed in the previous study, with participants counting FID units and calculating their prandial insulin doses using an individualised insulin: FID ratio.

After 12 weeks, changes in HbA1c were similar in both groups but the FID counters showed a trend to reduced risk of hypoglycaemia (-43% compared with baseline), inferring a trend towards improved glycaemic stability. As a pilot study, the results indicated that FID counting was at least as good as carbohydrate counting for maintaining glycaemic control, and therefore deserving of further research.

The potential of the FII to improve glycaemic control is not limited to type 1 diabetes. Type 2 diabetes is a metabolic disorder also characterised by chronic hyperglycaemia and similar long term complications. The pathogenesis differs because worsening insulin resistance and a relative insulin deficiency due to β -cell failure are the hallmarks of type 2 diabetes. In turn, chronic hyperinsulinaemia may exacerbate insulin resistance and β -cell failure. Dietary patterns characterised by high insulin demand are pertinent to the dramatic rise in type 2 diabetes over recent decades. Compared with traditional diets, modern carbohydrate foods, including potatoes, bread and rice are rapidly digested and absorbed, thereby increasing dietary glycaemic load, and the potential for disproportionate insulin secretion and insulin resistance. Therefore dietary strategies that reduce insulin demand, may help to improve metabolic risk and minimise the rate of diabetes complications. Because the FII ranks foods by relative insulin demand per unit of energy, it offers an alternate dietary approach to reducing postprandial hyperinsulinaemia. For this reason, the 5th and final study in this thesis was designed to explore the potential of the FII in type 2 diabetes by comparing the day-long plasma glucose and insulin responses of 10 adults with type 2 diabetes on a low-FII vs. a high-FII diet. As hypothesised, the low FII diet produced a significantly lower day-long insulin response compared to a high FII diet, even though they were matched for all macronutrients, fibre and GI. These findings suggest that the FII may be applicable to type 2 diabetes, even in those with severely compromised insulin secretion. Hence, FII represents a promising dietary strategy for reducing postprandial hyperinsulinaemia, thereby reducing insulin resistance and preserving β -cell function.

Collectively, these studies offer new insights into the relationship between food and normal physiological insulin secretion and the potential of the FII for optimising glycaemic control and managing both type 1 and type 2 diabetes.

7.2 Practical implications & directions for future research

The results of this thesis add to the growing body of evidence that supports the use of the FII in the clinical and dietary management of diabetes. Nonetheless, the concept is still in its infancy and requires further research before it could be integrated into clinical practice.

Testing more foods and beverages for their FII value is critical to the application of the FII concept. Expanding the current database will allow detailed exploration of the relationship between nutritional and metabolic factors and normal insulin demand. This will improve our ability to predict the insulinaemic responses to foods and can help guide nutrition recommendations. Furthermore, the FII is determined by the 120 min iAUC, as per the GI testing ISO, however potentially longer test sessions may be relevant for the FII, particularly for foods high in protein and fat. Therefore further studies with longer test sessions (3-5 h) are warranted to compare the iAUC.

The FII is determined in single foods, but in practice, foods are commonly consumed as part of a mixed meal. Chapter 3 explored the hypothesis that the observed insulin response cannot be predicted by the nutrient content of the food alone, potentially due to the metabolic and nutritional interactions between and within foods. Since only 50-60% of the variability could be explained by known factors, it could be argued that the FII is not relevant to mixed meals or chronic feeding because the interactions between combinations of different single foods are unknown. However, *in vivo* testing of the FII of 13 mixed meals showed the observed insulin response correlated strongly with calculated insulin demand predicted by the FII of the component foods ($r = 0.78$, $p = 0.0016$)⁴. Hence, the FII (and derived FID) appear to be relevant to estimating insulin demand and therefore insulin dose for mixed meals as well as single foods.

Furthermore, expanding the database is critical for the clinical implementation of the FII in order that the results can be translated into practical food choices. Some subjects in the FOODII Study (Chapter 5) commented that the limited number of foods with a tested FII value made some meal estimations difficult. This is an inherent limitation of FID counting as the FII of each food is determined from the postprandial insulin responses of lean, healthy adults and cannot be calculated from the macronutrient composition. Therefore, expansion of the FII database needs to continue before FID counting could become widely implemented.

Once the FII database is enlarged, the stage is also set for undertaking a larger, longer, adequately powered RCT of FII in type 1 diabetes. In retrospect, the pilot study (Chapter 5) should have been powered to detect differences in hypoglycaemic events because there was the potential to find more (rather than fewer) events in the FII arm. A 12-month trial of the FII in practice should therefore be conducted to confirm the trend for reduced time in the hypoglycaemic range. Furthermore, the research focus should be expanded to include those with type 1 diabetes using multiple daily insulin injections rather than an insulin pump as well as to children and adolescents with type 1 diabetes.

Despite the potential for improvements in glycaemic control, the FII algorithm has been criticised as too difficult and complex to be integrated into routine clinical practice in type 1 diabetes. However, our experience during the pilot study revealed this was not the reality (Chapter 5). All participants using FID counting appeared to adapt to the new system quickly and at the conclusion of the trial rated the method as “easy to use”, with just under half of subjects opting to continue with the system over carbohydrate counting if the option had been provided. However, changes in HbA1c may have been tempered in the FID counters as they were required to learn a novel dosing strategy and become proficient with a new method of counting in a short timeframe, while the carbohydrate counters simply revised their

knowledge and skills. In future studies, this adjustment period should be accommodated as a 1-2 month 'run in' period, followed by at least 3 months of routine use so that any changes in HbA1c can be detected.

Participants were offered a variety of teaching environments and resources which may have assisted with the adoption of the new algorithm, including both group and individual sessions with the dietitian as well as written and pictorial printed resources, a website and an iPhone app (see sample resources at the end of Chapter 5). FID counting is essentially no more difficult than carbohydrate counting in practice, with both systems relying on counting units (either carbohydrate or FID) in foods consumed and converting this to an insulin dose using a ratio. The drawback, of course, is that additional foods need to be considered when FID counting, and currently FID units are not printed on food packaging as is carbohydrate. The issue of food labelling can be overcome, to some degree, with the use of a phone app which has the FII of foods stored in a database and automatically calculates the FID for the selected food and portion size (based on energy density, which is available on food packaging and could be considered more accurate due to issues with accurately measuring available carbohydrate).

Additionally, the IFR of each participant was not titrated to their individual needs (it was simply calculated from their current ICR), and this may have attenuated any potential benefit of the FII. To overcome this issue, future studies could target newly diagnosed subjects with type 1 diabetes who have no prior knowledge of carbohydrate counting. A longer trial duration with frequent contact with health professionals, especially initially to assist with the mastery of the food counting system and the titration of the mealtime insulin ratio is recommended. These considerations will also be important for the implementation of the FID counting, or any other new system of estimating insulin doses, in clinical practice.

Although there was a trend toward increased risk of hypoglycaemia with the FII compared to carbohydrate counting in Chapter 4, the opposite trend was seen in Chapter 5, with a 43% reduction in the risk of hypoglycaemia over the 12-week trial duration. An early study of the FII also revealed improvements in postprandial glycaemic control after mixed breakfast meals with no increased risk of hypoglycaemia³. Given the risks of unstable glycaemia in contributing to oxidative stress and diabetes complications^{5, 6}, these trends warrant further research to clarify the full picture in order to identify the possibilities for the FII to improve glycaemic control and the opportunities to further improve the algorithm. To do so, studies are needed that are specifically designed and powered to detect the risk of hypoglycaemia in type 1 diabetes, including both acute and chronic feeding studies. Higher baseline fasting blood glucose levels and day-long testing sessions are needed to glean more information about the risk of hypoglycaemia.

It is also possible that the observed trend towards hypoglycaemia seen in Chapter 4 may be related to the types of foods and insulin bolus delivery pattern used in the study design. The normal wave bolus used for all test sessions may not closely follow the normal postprandial physiological insulin profile for high protein and/or fat foods. The standard (or 'normal-wave') insulin delivery system has been developed to deal with high carbohydrate meals, with the insulin dose delivered rapidly resulting in increased risk of hypoglycaemia if the meal is lower in carbohydrate than expected. Previous research has highlighted the benefit of dual-wave or square insulin bolus delivery patterns in the case of higher fat, higher protein and lower GI meals. In this instance, only a proportion of the total insulin dose is delivered immediately (often 50-70%) and the remainder delivered slowly over an extended period of time⁷⁻¹⁰. Thus, further research should test the hypothesis that the optimal bolus insulin pattern for glycaemic control for foods of varying FII may be either square or dual-wave.

The FII concept also showed promise for reducing postprandial hyperinsulinaemia in the management of type 2 diabetes (Chapter 6). This study was conducted in overweight adults who did not require insulin to manage their diabetes. Thus the utility of this dietary strategy should be further explored in type 2 adults with little residual pancreatic β -cell function. Indeed, further research is also warranted to determine if a low FII diet can produce clinically meaningful improvements in metabolic control in free-living type 2 individuals over a longer period of time.

The FII has the potential to change the nutritional management paradigm in both type 1 and type 2 diabetes. Current dietary management focuses heavily on carbohydrate intake for managing postprandial blood glucose levels, yet meta-analysis has shown this may not be an effective strategy and there is evidence it can foster unhealthy eating patterns and food beliefs. Carbohydrate counting has been linked with unhealthy food beliefs, an increased reliance on packaged foods (as carbohydrate content is listed) and high intakes of fats and protein exceeding nutritional recommendations. Studies have shown that children and adolescents with type 1 diabetes are at greater risk of atherosclerosis, dyslipidaemia and CVD, possibly due to an avoidance of carbohydrate and insulin, and higher intake of saturated fat. Alternatively, the FII represents a more holistic and practical approach to diabetes management that focuses on whole foods, rather than specific macronutrients within foods. Unlike carbohydrate, the FID values are not available on packaged foods and therefore the FII could reduce the reliance on packaged foods reported with carbohydrate counting. Nonetheless, it will always be important to consider not just the overall insulin demand of the meal or overall diet but also the balance, quality and quantity of food consumed to achieve an enjoyable, healthy and nutritious diet.

7.3 Conclusion

The achievement of optimal glycaemic control is critical to the management of diabetes in order to minimise the risk of acute and chronic complications. Yet, in practice, it remains difficult to achieve. Even patients within target HbA1c levels continue to experience unanticipated hyper- and hypoglycaemia, particularly in response to meals high in protein and/or fat¹¹⁻¹⁴. The studies presented in this thesis build the evidence base for the FII as a promising tool for optimising glycaemic control in individuals with diabetes. Until a cure for diabetes can be found, any clinically significant advance in glucose control and reduced glycaemic variability offer people with diabetes greater wellbeing through a reduced burden of disease and decreased risk of long-term diabetes-related complications.

7.4 References

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