Understanding and Diagnosing Hyperinsulinaemia

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Abstract

Traditionally, insulin resistance is thought to be the precursor to many metabolic diseases. It is now believed that compensatory hyperinsulinaemia, previously thought to be a symptom of insulin resistance, may independently associated with metabolic disease and have its own pathological implications. Further understanding of compensatory hyperinsulinaemia may offer new insights into the aetiology of metabolic disease.

This thesis provides novel work in hyperinsulinaemia and is broadly divided into four parts. Part 1 comprises a collation of the literature to show the aetiology and pathologies of hyperinsulinaemia, and to critically review the current diagnostic methods. The aetiology of hyperinsulinaemia is not yet fully elucidated, but is likely to include excessive carbohydrate ingestion, excessive cortisol or uric acid production, and/or medications. Subsequent pathologies include: cardio-, cerebro-, and peripheral-vascular disorders; type 2 diabetes; inflammation; and certain cancers or dementias. This is the first review to comprehensively link hyperinsulinaemia to such a wide range of metabolic disorders. Except for fasting insulin levels being considered unreliable, there was no consensus regarding diagnostic criteria. This means that diagnostic criteria needs to be determined prior to further research.

Part 2 examined the prevalence of hyperinsulinaemia in the Kraft database. This important database comprises a large sample of oral glucose tolerance tests with insulin assays collected over 20 years in Chicago, USA. From the 15 000 available tests, those involving men aged ≥ 20 years and women ≥ 45 years, with a BMI > 18kg/m^2 were included (n = 7750). Participants were stratified according to glucose response (WHO criteria) and insulin response according to the Kraft (2014) response patterns. The results showed that > 90% of people with diabetes or impaired glucose tolerance were hyperinsulinaemic. Of those with normal glucose tolerance (n = 4030), approximately 75% were hyperinsulinaemic. This had a limited association with obesity. As this is the first time a cohort of people with normal glucose tolerance have had their insulin response patterns analysed, these results show that there may be high prevalence of hyperinsulinaemia in the wider community. As this was not associated with obesity, this implies that hyperinsulinaemia is a silent disease. Together with the implications of the potential pathologies resulting from hyperinsulinaemia, there is a need for a robust

diagnostic test. These results are important because it is the first time the potential impact of hyperinsulinaemia in the wider community has been investigated.

Part 3 investigates which test(s) could best be used for diagnosing hyperinsulinaemia. Chapter 4 investigates whether the existing insulin resistance tests, which include the homeostasis model assessment variants (HOMA2 %B, %S, and IR), and the oral glucose insulin sensitivity (OGIS) have sufficient test-retest reliability to be considered as a potential diagnostic test. Using the methods of Bland and Altman, the test-retest reliability was calculated as $Test\ 1\ \approx Test\ 2\ \pm repeatability\ coefficient$, while the repeatability coefficients were derived from the square root of the residual mean square errors from one-way analyses of variance. This is the first time repeatability coefficients have been calculated for these variables with potentially higher practical utility compared to coefficient of variation. The results showed that the repeatability coefficients for the HOMA2 %B, %S, and IR variants were 72.91, 189.75, and 0.9, which equated to 89%, 135%, and 89% of their respective grand means. OGIS had a repeatability coefficient of 87.13 which equated to 21% of the grand mean. These finding are important as they demonstrate that dynamic measures should be preferred to fasting measures when assessing either insulin resistance or hyperinsulinaemia. These results also question the validity of the widespread use of HOMA.

There was no test-retest repeatability data for either the Kraft or Hayashi insulin response patterns and a limited amount for the McAuley Index, another measure of insulin resistance. Therefore, Chapter 5 reports on three-hour, 100 g, oral glucose tolerance tests with insulin assays that were conducted four times on six healthy individuals at weekly intervals. Test-retest repeatability assessments were conducted as according to the methods previously described for measures of insulin resistance (HOMA2 variants, OGIS, and McAuley Index), while Fleiss' kappa was applied to Kraft and Hayashi dynamic insulin response patterns. The results showed that Kraft patterns had a higher repeatability compared to Hayashi patterns based on a combination of Fleiss' kappa (0.290 vs 0.186,) *p*-value (0.15 vs 0.798) and 95% confidence intervals. OGIS and McAuley index recorded a lower CV compared to HOMA2 variables. However, the McAuley index was unable to distinguish between people with normal or a hyperinsulinaemic response, suggesting a low-overall sensitivity.

These results show that a dynamic insulin response following an oral glucose load is needed to effectively diagnose hyperinsulinaemia. However, this requires a minimum of three blood tests over a minimum of three hours test duration. In Chapter 6, the Kraft database was re-examined to determine whether a simplified diagnostic algorithm could be derived. In people with normal glucose tolerance and fasting plasma insulin < 30 μU/mL, sensitivity and specificity calculations showed that hyperinsulinaemia can be diagnosed by 2-hr plasma insulin (> 30 μU/mL sensitivity/specificity = 0.98/0.62; > 50 μU/mL sensitivity/specificity = 0.79/0.99). Given that first-line treatment for hyperinsulinaemia is lifestyle management, the lower level of > 30 μU/mL was recommended as the new diagnostic criteria for hyperinsulinaemia.

The fourth part of this thesis reviewed potential treatment options for hyperinsulinaemia including pharmacotherapy, physical activity and diet. Hyperinsulinaemia cannot be managed without concurrent management of glycaemia; thus limiting pharmaceutical agents. Physical activity, especially high intensity interval training in combination with resistance training, and dietary management, especially carbohydrate restriction, appear to offer the most promise, and may even work synergistically.

Overall, this thesis represents new knowledge in examining hyperinsulinaemia; from aetiology to management. Hyperinsulinaemia contributes to a significant number of metabolic diseases, including cancer and dementia. Hyperinsulinaemia affects almost every person with a glucose tolerance disorder and many people with normal glucose tolerance; but is not associated with obesity. Repeatability coefficient testing determined that dynamic measures should be preferred over fasting for both insulin resistance and hyperinsulinaemia. Following a 100 g, oral glucose tolerance test, 2-hr insulin > 30 μU/mL is diagnostic for hyperinsulinaemia. Public health clinicians and researchers can build on these foundations to determine further means of stemming the tide against metabolic disease.

Contents

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material to which a substantial extent has already been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Catherine Crofts

Co-Author contributions

Chapter 2: Hyperinsulinaemia: A Unifying Theory of Chronic Disease?

Catherine Crofts: Substantial contributions to concept, literature searching and selection, and manuscript drafting (80%)

Caryn Zinn: Contributions to final section of literature and critical revisions (5%)

Mark Wheldon: Contributions to critical revisions (5%)

Grant Schofield: Contributions to manuscript concept, final section of literature and critical revisions (10%)

Chapter 3: Identifying Hyperinsulinaemia in the Absence of Impaired Glucose Tolerance: An Examination of the Kraft Database.

Catherine Crofts: Substantial contributions to manuscript concept, design, data analysis and interpretation. Drafted the article and performed data and statistical analysis. (75%)

Grant Schofield: contributions to manuscript concept, design, data interpretation and critical revisions. (5%)

Caryn Zinn: contributions to manuscript concept, design, data interpretation and critical revisions. (5%)

Mark Wheldon: Substantial contributions to manuscript design, data analysis and interpretation, and critical revisions. (5%)

Joseph R Kraft: Data collection, initial pattern development (1975), and contributions to manuscript drafting and data analysis. (10%)

Chapter 4: HOMA: Too blunt an instrument?

Catherine Crofts: Substantial contributions to manuscript concept, design, data analysis and interpretation. Drafted the article and performed data and statistical analysis. (75%)

Mark Wheldon Substantial contributions to manuscript design, data analysis and interpretation, and critical revisions. (10%)

Caryn Zinn contributions to manuscript design and critical revisions (5%)

Thomas Wolever Data collection and final editing of manuscript (2.5%)

Xiaomiao Lan-Pidhainy Data collection and final editing of manuscript (2.5%)

Grant Schofield: contributions to manuscript concept and critical revisions (5%)

Chapter 5: Dynamic insulin responses are superior to fasting measures for diagnosing insulin resistance or hyperinsulinaemia

Catherine Crofts Substantial contributions to study concept and design, data collection, analysis, and interpretation. Drafted the article and performed data and statistical analysis. (80%)

Caryn Zinn Contributions to study design and critical revisions to manuscript (5%)

Mark Wheldon Contributions to study design, statistical analysis and data interpretation. Critical revisions to manuscript (10%)

Grant Schofield Contributions to study design and critical revisions to manuscript (5%)

Chapter 6: Determining a diagnostic algorithm for hyperinsulinaemia

Catherine Crofts Substantial contributions to study concept and design, data collection, analysis, and interpretation. Drafted the article and performed data and statistical analysis. (80%)

Grant Schofield contributions to manuscript concept and critical revisions. (5%)

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Chapter 7: Hyperinsulinaemia: Best management practice

Catherine Crofts: Substantial contributions to concept, literature searching and selection, and manuscript drafting (80%)

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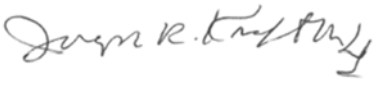
Joseph R Kraft 

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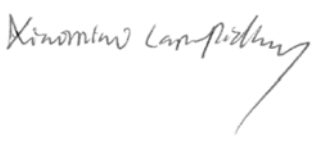
Xiaomiao Lan-Pidhainy 

List of Publications Arising from Doctoral Thesis

Papers published

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Papers in submission

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Papers in preparation for submission

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Crofts, C., Wheldon, M., Zinn, C., & Schofield, G. (in draft). Assessing the repeatability characteristics of insulin response patterns and measures of insulin resistance.

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Dedication

In loving memory of my mother

Wendy Price
(1949-2006)

Had we but known about the impact of insulin on cancer.

Chapter 1: Introduction

Background

Context

The increasing prevalence of non-communicable diseases has been described as a "global crisis". These diseases, which include type 2 diabetes, all forms of vascular disease, cancer, osteoarthritis and dementia, affect all people, of all ages, across all income groups in both developing and developed countries. Progresses in economic development, health, and living standards are under threat due to increasing morbidity and/or early mortality (Beaglehole et al., 2011). Globally, two out of every three deaths can be attributed to a non-communicable disease, with a third of these deaths occurring in people aged less than 60 years. Of these deaths, 80% are in low-to-middle-income countries, which must also contend with the longstanding challenge of infectious disease. This double burden places enormous strains on under-resourced health systems (Beaglehole et al., 2011).

In New Zealand, non-communicable diseases are a considerable health burden (Ministry of Health, 2003, 2012b). Ischaemic heart disease is the leading cause of death, (as ranked by both age-standardised mortality rates and years of life lost,) for both men and women of all ethnicities. Other non-communicable diseases complete the five major causes of death for both Maori and non-Maori women, while men are considerably affected by suicide (Maori and non-Maori) and motor-traffic accidents (Maori only) (Ministry of Health, 2012b).

A further global pressure resulting from non-communicable disease is the financial burden placed on the individual and their family. These may include the direct financial cost of health care, such as medications, or hospitalisation, and the indirect costs of ill-health including loss of income (both the patient and family carer/s), transportation to health-care appointments, and managing labour substitutions within the household (Kankeu, Saksena, Xu, & Evans, 2013; Siegel & Narayan, 2008). This reduced well-being and increased financial vulnerability may have long-term ramifications. For example, men with a previously clinically diagnosed myocardial infarction have a 50% chance of surviving another ten years (Lampe et al., 2000) but may not return to their previous quality of life (Mark et al., 1994).

As a pharmacist, I know we should be concerned about these increasing rates of disease as I work directly with patients and their families. When I commenced practice in Auckland in 1997, people with type 2 diabetes were typically over the age of 40 years and more likely to be in their mid-60s. I distinctly remember being presented with the, 'rare' case of someone in their mid-20s with type 2 diabetes. At the time, around 1 in 27 adults were diagnosed with diabetes (Statistics New Zealand, 1997). This prevalence differs with sociodemographic factors. Increased prevalence of diabetes was associated with increasing age, increasing community deprivation index and decreasing family income and with Maori and Pacific Island ethnicities.

These days, it is more common to see a much younger population presenting with type 2 diabetes, with increasing numbers of teenagers, and even younger children. Over the years, these children will need more medication to maintain current standards of living and, compared to their peers, they will have a poorer quality of life. From my perspective, it seems that more of my patients are presenting with chronic disease pathologies, which, at best, can only be controlled by medications. The majority do not enter a remissive state with lifestyle modification. It just seems wrong to tell increasing numbers of younger adults that they need to take medication for the rest of their lives for a chronic metabolic disease. There are times that I feel that I am simply a "disease-management" specialist rather than a "health-care" professional. My patients and I all agree that preventing metabolic disease is far preferable to treatment.

Of concern, is that many of my patients are well-educated and trying to adhere to public health guidelines. With the increasing prevalence of obesity and metabolic disease since the 1970s in both developed and developing countries, I think we can safely assume that current public health measures are unsuccessful in preventing metabolic disease.

When I considered all the common medication that my patients were taking for metabolic disease, there were common themes around managing blood pressure, cholesterol and blood glucose. These are all conditions that, theoretically, could be managed with lifestyle modification, but in practice, very few achieved control this way; hence medication management. Patients would tell me that they were adhering to their diet and exercise programs, but I watched their health deteriorate over the years. Either my patients were not being honest about their adherence or "best-practice" only gave them a medication-free lifestyle for a relatively short period of time.

What is apparent, is that many of the medications that these people were taking were there to manage a condition known as metabolic syndrome; an aggregation of symptoms including obesity, impaired glycaemic profile, dyslipidaemia, and hypertension. It is a multifaceted clinical entity resulting from a combination of genetic and lifestyle factors (Boehm & Claudi-Boehm, 2005). If I was to understand how best to optimise my patients' medications, it was clear that I first needed to have a thorough understanding of metabolic syndrome.

This cluster of symptoms known as metabolic syndrome is underpinned by a condition known as insulin resistance and each of the main symptoms of metabolic syndrome can trace insulin resistance as part of its aetiology, or set of causes. But as my thesis will demonstrate, there is no mechanistic pathway that directly aligns the insulin resistance with the subsequent pathologies of metabolic disease. What has become clear from the literature is that hyperinsulinaemia, or chronically high levels of insulin, almost always coexists with insulin resistance, with some papers practically referring to them as an interdependent state. Hyperinsulinaemia could also plausibly explain some of the mechanistic pathways that led from a healthy person, to someone with a high degree of morbidity due to metabolic disease. But there was a paucity of information on the topic.

From my initial investigations, I believe that we have some substantial gaps in basic knowledge regarding these metabolic diseases, especially with respect to causality. An emerging theory is that hyperinsulinaemia, may be a risk factor for other, known, risk factors for metabolic diseases, including obesity or dyslipidaemia. Therefore, monitoring insulin levels may be the earliest marker for metabolic disease. Yet, there are no recommended reference ranges, or standardised testing protocols for insulin. In short, we still don't understand what constitutes a "normal" or "abnormal" insulin response. Another challenge with insulin is that it is extremely labile, due to a pulsatile secretion and short half-life. The first stage to being able to understand if hyperinsulinaemia can be used as a marker of metabolic disease risk is to determine a testing protocol and a "normal" insulin response range.

Thesis rationale

Statement of the problem

Non-communicable diseases including cardiovascular disease, type 2 diabetes, cerebrovascular disease and certain cancers are amongst the major causes of death, ranked by both age-standardised mortality rates and by years of life lost (Ministry of Health, 2012a). These diseases, along with dementia, also have a significant economic and social impact. Metabolic syndrome and/or type 2 diabetes are considered predictors of these pathophysiologies. Although insulin resistance is considered to underpin the symptoms of metabolic syndrome, diagnosing insulin resistance is expensive and does not change clinical treatment options. Furthermore, the diagnosis of insulin resistance does not enhance disease risk prediction calculators. Instead, a person is generally diagnosed with insulin resistance after they have presented with hyperglycaemia or other symptoms of metabolic syndrome. This may be too late to prevent pancreatic β-cell attrition or other end-stage disease processes.

Insulin resistance can be defined as the cell's inability to take up glucose. Simply impeding the glucose uptake rate cannot mechanistically explain many of the pathophysiological changes associated with metabolic syndrome. However, when a person with insulin resistance is subjected to a glucose load, they will become hyperinsulinaemic – a higher than expected insulin response. This hyperinsulinaemia may occur with an apparently normal glucose response and may explain the pathophysiological changes associated with metabolic syndrome and subsequent disease states. Current medical practice does not include diagnosis or monitoring of hyperinsulinaemia, and therefore, little is known about the condition.

Due to the continued focus on insulin resistance and metabolic disease, there is a paucity of data regarding whether hyperinsulinaemia should be considered a health risk independent to insulin resistance. On a general level, it is not yet known the extent of the population who may be affected by hyperinsulinaemia, or even which non-communicable diseases that are caused by and/or aggravated by hyperinsulinaemia. Alternatively, on the individual level, the features such as age or obesity, which may characterise the hyperinsulinaemic individual also remain unknown. If hyperinsulinaemia is identified as being potentially associated with either a large proportion of the population and/or non-communicable diseases that are known to be highly prevalent in the population, then a standardised test should be considered.

Currently, there is no standardised test for hyperinsulinaemia. It is plausible that tests for insulin resistance may be able to be used to assess hyperinsulinaemia, as there is a degree of agreement that the two conditions are intertwined.

This thesis will be one of the first series of studies that will tie together what is currently known about hyperinsulinaemia, assess whether it should be considered an independent health risk, and recommend a diagnostic/monitoring test. This will provide a base for both future research and/or clinical investigations into the reducing the effects of hyperinsulinaemia on population health.

Statement of purpose

The overarching aim of this research is to understand the fundamental principles on which further research into hyperinsulinaemia may be based. Specific aims include:

1. Review and critique existing literature (Chapter 2) that:
    a. Examines the pathophysiology of metabolic syndrome, with reference to the wider epidemiological field.
    b. determines whether hyperinsulinaemia should be considered separate to insulin resistance by examining the aetiology, physiology, resultant pathophysiologies, and diagnostic techniques of hyperinsulinaemia.

2. Quantify and describe the insulin response characteristics of two different population cross-sections (Chapter 3):
    a. The general population segmented according to World Health Organization (WHO) glucose impairment disorder criteria.
    b. Individuals with normal glucose tolerance segmented according to insulin response patterns.

3. Examine a common fasting and dynamic technique for quantifying insulin resistance and determine whether these tests are sufficiently sensitive for clinical use and should be considered for the diagnosis and management of hyperinsulinaemia (Chapter 4).

4. Assess the repeatability of two different insulin response patterns and compare these to the repeatability of other indices of insulin resistance (Chapter 5).

5. Determine a novel approach to the diagnosis of hyperinsulinaemia that could be applied to both research and healthcare practice (Chapter 6).

Since many health professionals would believe it unethical to identify a pathophysiological state without a viable management strategy, another specific aim of this thesis is:

6. To review existing literature to determine potential strategies to prevent, or mitigate, the effects of hyperinsulinaemia (Chapter 7).

Significance of the research

The series of studies within this thesis contains several novel contributions to the body of literature, predominantly for public health and disease prevention. These studies though, may also aid in devising alternative treatment strategies for many different non-communicable diseases that have their roots in metabolic syndrome.

It is likely that everyone will be affected in some way by non-communicable diseases. The majority of us will be directly affected by one, or more, non-communicable diseases, while the remainder will have family members affected, or be required to shoulder some of the societal burden of disease. Most of these diseases cannot be cured. The best that can be hoped for is to slow the rate of progression and maximise quality of life. When this definition is applied to cancer treatment, it is described as 'palliative care'.

If hyperinsulinaemia is indeed a root cause for many non-communicable diseases, then further understanding of the condition is highly significant as it may enable novel pathways for prevention or mitigation strategies. The first step in understanding a condition is to have a valid measurement tool. There is a paucity of literature on available tools for assessing hyperinsulinaemia. What is known is unsatisfactory: The limitations to fasting insulin are recognised while the other widely available measures focus on insulin resistance. Very few studies evaluate the use of insulin response patterns. My thesis will evaluate and investigate available tools, and make a recommendation as to which tool should be used in future research and/or clinical practice to diagnose and/or monitor hyperinsulinaemia to understand disease progression and/or clinical interventions.

Study delimitations

Parameters specific to this body of work are as follows:

1. One aim of this thesis was to understand who is affected by hyperinsulinaemia. The complete Kraft database, from which my study data was derived, included information from a very wide sampling of the community. Participants were not excluded on the basis of age, gender, or health status (including pregnancy). Ethnicity was not recorded. For the purposes of this study, we deliberately

excluded some sub-groups due to potential confounding health conditions. Two significant groups affected were all people aged less than 20 years and women aged less than 45 years. The children and young adults were excluded as the effects of growth on hyperinsulinaemia has yet to be determined. These children and young adults also received a glucose dose that was based on weight. The variation of this dose on subsequent insulin response is unknown. Pregnancy aggravates insulin resistance, thus influencing hyperinsulinaemia (Barbour et al., 2007). Therefore, all women under the age of 45 years were excluded as it could not be established from the information provided whether or not they were pregnant. These delimitations were believed to be a balance between excluding people with potentially confounding conditions and including as many participants as practical.

2. Normal protocol for oral glucose tolerance tests is to omit, on the morning of the test, all normal medication that may influence the test. This does not always occur in practice. Results that had an overly exaggerated insulin response were excluded from analysis on the assumption that the participant had consumed their normal medication. This assumption may lead to an underestimation of the proportions of individuals affected by hyperinsulinaemia.

3. Results from the Kraft data base are derived from a 100 g oral glucose tolerance test that was standard protocol at the time at St Joseph's Hospital. Subsequently, the WHO recommended that the glucose load be standardised at 75 g. Furthermore, the nominal blood glucose values for diagnosing diabetes based on the oral glucose tolerance test have been redefined on more than one occasion since the 1970s (William H. Herman, 2007). There is little data available to determine whether the same nominal blood glucose values for diagnosing diabetes can be used for the 75 g and 100 g oral glucose loads. Therefore, this thesis employs the same WHO criteria for defining glucose tolerances for both 75 g and 100 g oral glucose loads for two main reasons. The American Diabetes Association recommends the same blood glucose values be used for diagnosing gestational diabetes when either the 75 g or 100 g glucose loads are used (2010). Dr Kraft employed current American Diabetes Association criteria for defining normal and impaired glucose tolerances in his data analysis (2011).

4. This thesis is focussed on the diagnosis of hyperinsulinaemia. The data collected for each study was cross-sectional. No data was gathered either prior to, or following the test protocol. While this would have been ideal as it would both further understanding the causality of and outcomes resulting from hyperinsulinaemia, this information is not available. Therefore, neither causality nor outcomes can be inferred.

Thesis overview

Thesis organisation

This thesis is presented as a sequential progression of studies arranged in a series of chapters as shown in Figure 1**Error! Reference source not found.**. Chapter 2 provides the context as to why hyperinsulinaemia should be considered an independent health risk and the challenges surrounding diagnosis. Chapter 3 analyses the Kraft database and ascertains that a significant proportion of hyperinsulinaemia occurs in the general population, especially hyperinsulinaemia in the presence of normal glucose tolerance. Chapter 4 considers whether the insulin resistance models are sufficiently sensitive to be considered potential hyperinsulinaemia clinical diagnostic tools. Findings from these latter two chapters determined the need to assess the test-retest repeatability of insulin response patterns. Therefore, Chapter 5 is an assessment of the test-retest repeatability of insulin response patterns and simple indices of insulin resistance. Having assessed that a dynamic insulin response is superior to fasting methods for assessing hyperinsulinaemia, Chapter 6 uses sensitivity and specificity calculations to determine whether there is a simpler method of diagnosing hyperinsulinaemia. As prescribers and other diagnosticians are often reluctant to test for a clinical condition if there is no viable management strategy, Chapter 7 critically examines the literature to provide a theoretical framework on which treatment decisions can be made. The purpose of Chapter 8 is to bring together the findings and recommendations that emerged from the research and the implications of these in the research and wider communities while also noting the research limitations. Chapters 2 to 7 inclusive have been prepared as, or adapted from, papers to be published in peer-reviewed journals. As such, repetition of information may occur. Chapters are prefaced by linking information that outline the logical progression of the thesis as a whole. Supplementary information not provided in the thesis chapters are included as Appendices.

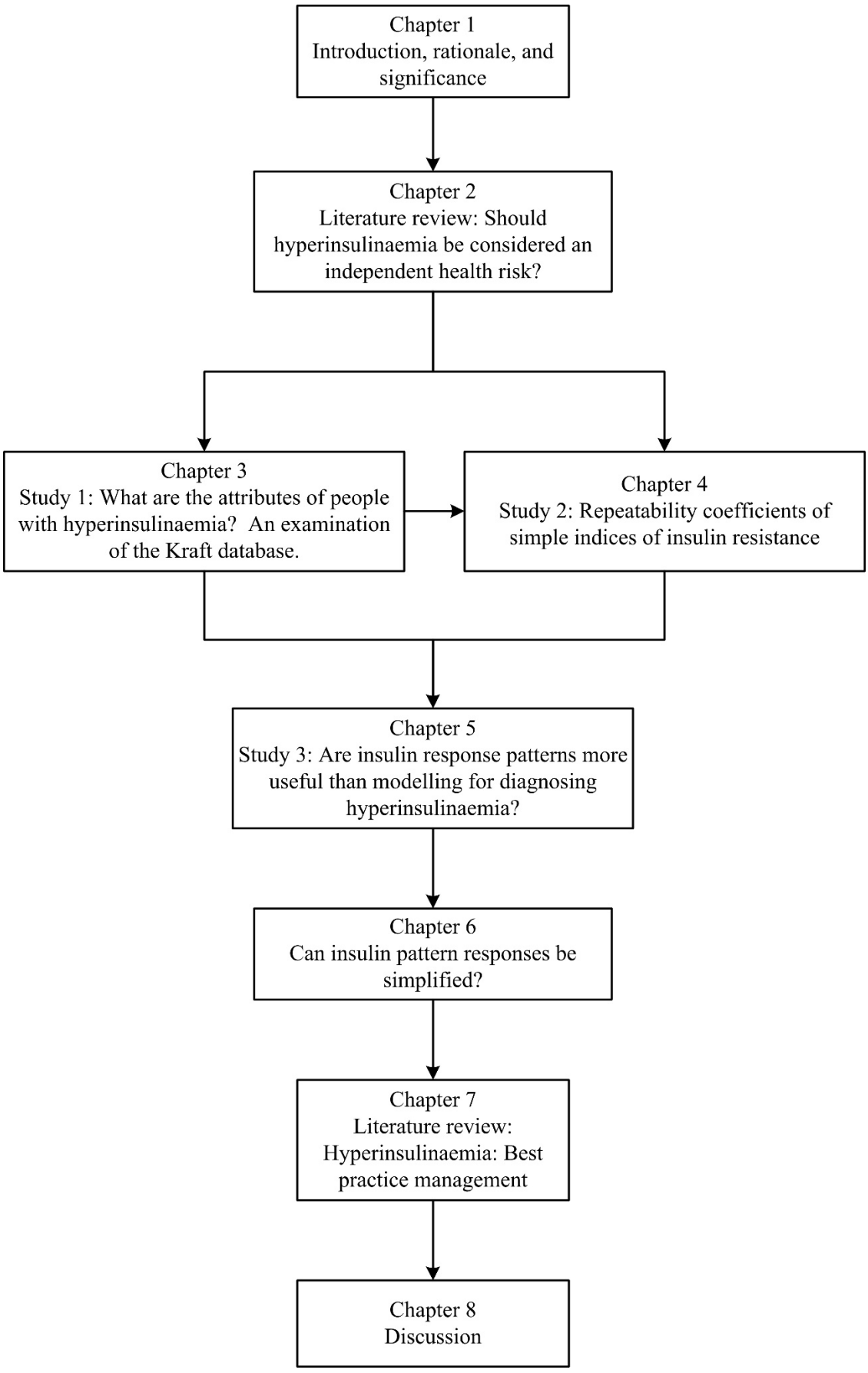

Figure 1: Thesis structure

Thesis methodology

One strength, and weakness, of this thesis is its heavy reliance on secondary analysis of previously collected data; of my four original investigations, three are based on secondary analysis. There are a number of advantages to secondary analysis. For example, the 15,000 patient records in the Kraft database was collated over approximately 20 years using routine medical procedures. To try and collect the same wealth of data today would be impractical. For starters, this would conservatively cost more than $400,000 simply for blood analysis alone - excluding wages for the phlebotomist, and the laboratory technicians. It would also take an extensive period of time to collect the data, potentially years considering the original data was collected over a 20-year period. The resultant costs and time requirements to re-collect this data would not occur within the constraints of a PhD.

The question of ethics must also be asked. Is it actually ethical to spend time and money, plus impose an unnecessary and invasive, and potentially risky, procedure upon volunteers for little benefit except that to science? Especially when that data may already have been collected.

It must be noted that I am not repeating previously published studies. My analysis of Drs Lan-Pidhainy and Wolever's data was not one that they had originally envisaged. The original purpose of their data was to assess the glycaemic and insulinaemic testing of different foods. With respect to the Kraft database, while some of my analysis overlaps with that of Dr Kraft, his later work was never peer-reviewed. I am also extending his work by incorporating factors such as body mass index, and performing sub-analyses such as evaluating people with normal glucose tolerance as a separate group.

The counter-argument, and a potential weakness of my thesis, is, by not relying on primary data there is the potential to miss out on a fundamental part of the research process; that of understanding the data collection process. One original investigation in my thesis is based on data that I have personally collected, thereby fulfilling that need.

Chapter 2: Hyperinsulinaemia: A unifying theory of chronic disease?

Preface

The overarching aim of this thesis is to determine whether there is a viable way to detect people at high-risk of developing metabolic disease at an earlier stage than currently known. People with metabolic syndrome are at an increased risk of many other non-communicable diseases, therefore understanding the aetiology of metabolic syndrome may lead to a new research approach.

The purpose of this chapter is to explore the aetiology of metabolic syndrome focusing on insulin resistance and compensatory hyperinsulinaemia as described by Reaven (1988). While there is an abundance of literature on insulin resistance and associated pathologies, there is a paucity of literature that specifically references the compensatory hyperinsulinaemia. One systematic review collates the relationship of hyperinsulinaemia with other metabolic disorders (Kelly et al., 2014), but not other conditions. To my knowledge, there is no review or other summary of the aetiology, and pathophysiologies resulting from hyperinsulinaemia. Therefore, this chapter fulfils this need by providing a comprehensive summary of the aetiology, pathophysiology and diagnostic challenges of compensatory hyperinsulinaemia. This chapter is adapted from a stand-alone peer-reviewed paper, published by Diabesity (Crofts, Zinn, Wheldon, & Schofield, 2015) (Appendix A). The introduction was expanded to provide an initial account of metabolic syndrome to provide broader context.

For this narrative review chapter, literature was reviewed on hyperinsulinaemia and insulin resistance, targeting full-text English language studies. There was no date criterion. Articles were selected on the basis of having a minimum of both a plausible biological mechanism and established clinical association. Initially, the academic database search included EBSCO, Medline and Google Scholar, using variants of the terms “hyperinsulinaemia,” “insulin resistance,” “metabolic syndrome,” and “syndrome x,” individually and conjunction with “non-communicable disease,” “mechanism,” “atherosclerosis,” and “cardiovascular disease.” As subsequent metabolic diseases and/or mechanisms were eluded to in the initial search, search terms were widened so that no disease state was excluded. Subsequent metabolic diseases included, but were not limited to, conditions such as "non-alcoholic fatty liver disease,"

"cancer," "dementia." The final selection of references was based on the authors' judgment of relevance, completeness, and compatibility with clinical, epidemiological, pathological and biochemical criteria.

Abstract

Globally, there is an increasing prevalence of non-communicable diseases. The morbidity and mortality from these conditions confer a greater economic societal burden. Epidemiological research associates insulin resistance in the aetiology of these diseases, but there is limited evidence for the mechanism of damage. Emerging research suggests that hyperinsulinaemia, a symptom of insulin resistance, may cause these pathological changes, and therefore be an independent contributor to these diseases. This review shows that hyperinsulinaemia, or excessive insulin secretion, should be considered independently to insulin resistance, defined as glucose uptake rate, even though the two conditions are intertwined and will co-exist under normal conditions.

Hyperinsulinaemia directly and indirectly contributes to a vast array of metabolic diseases including all inflammatory conditions, all vascular diseases, gestational and type 2 diabetes, non-alcoholic fatty liver disease, obesity and certain cancers and dementias. The mechanisms include increased production of: insulin growth factor-1; reactive oxidative species and advanced glycation end-products; and triglyceride and fatty acids. Hyperinsulinaemia also directly and indirectly affects many other hormones and cytokine mechanisms including leptin, adiponectin and oestrogen.

There is limited research standardising the hyperinsulinaemia diagnostic process. Methodological concerns and lack of standardised reference ranges preclude the use of fasting insulin. Most research has also focused on insulin resistance and it is unknown whether these methods translate to hyperinsulinaemia.

Introduction

Metabolic syndrome

Globally there is an increase in non-communicable diseases in both developed and developing countries. While the term "non-communicable disease" can be interpreted to describe any non-infectious pathological state, the World Health Organization (WHO) generally considers non-communicable diseases (also known as NCDs) to have a slow progression and long duration (World Health Organization, 2015). The four main types of non-communicable diseases are cardiovascular diseases (including stroke), chronic respiratory disease (e.g. asthma), diabetes mellitus (commonly known simply as "diabetes", but distinct from diabetes insipidus), especially type 2 diabetes, and cancer (World Health Organization, 2015). Not included in these top four diseases, but of concern due to both its increasing prevalence and high morbidity, are dementias, especially Alzheimer's disease (Ferri et al., 2005). In New Zealand, these non-communicable diseases are estimated to account for 91% of all deaths as depicted in **Error! Reference source not found.**.

Although diabetes only comprises 3% of these deaths the global increase in diabetes

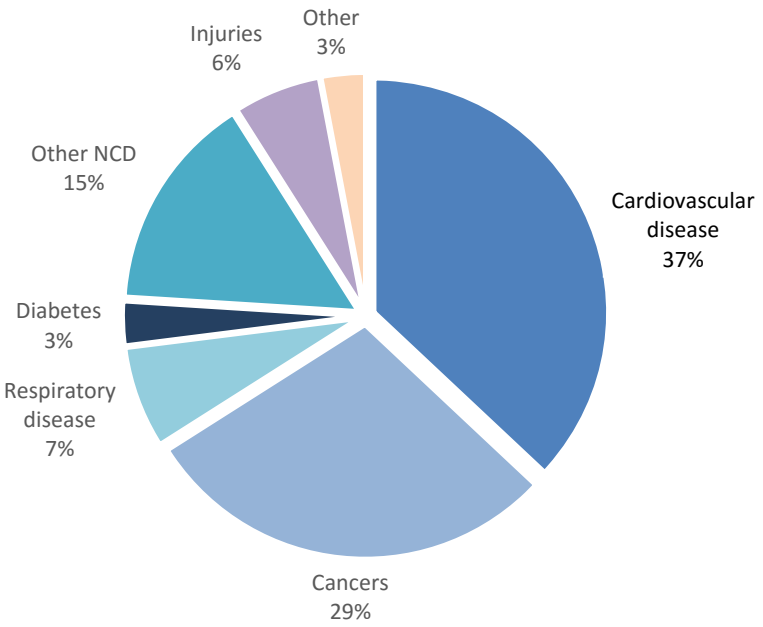

Figure 2: Proportion of all-age deaths in New Zealand, 2008, by primary cause (World Health Organization, 2011).

prevalence is of concern. Mortality associated directly with diabetes is generally that of diabetic ketoacidosis, and hypoglycaemia (Daneman, 2001). However, a diagnosis of diabetes, especially type 2 diabetes, is recognised to essentially double mortality risk

compared to the general population, with cardiovascular disease comprising the principal cause of death (Nwaneri, Cooper, & Bowen-Jones, 2013). However, those with diabetes are also known to be at risk of other vascular diseases including stroke, dementia, retinopathy, nephropathy and peripheral vascular diseases (Nathan, 1993). Diabetes is also associated with cancer incidence (Giovannucci et al., 2010). Taken together, having diabetes significantly increases the risk of developing other NCDs.

Although it was previously known that having diabetes increased the risk of later developing cardiovascular disease, it was not until 1988 when the two conditions were recognised to share a common aetiology of resistance to insulin-mediated glucose disposal and compensatory hyperinsulinaemia (Reaven, 2002). People with this condition, which became more simply known as, "insulin resistance" also tended to have a cluster of other metabolic symptoms that taken together increased the risk of metabolic disease.

There is a significant body of research into this condition, now commonly known as metabolic syndrome. However, because there are a minimum of three main definitions for metabolic syndrome as shown in Table 1, the results from this research need to be interpreted with caution.

Although there is a considerable degree of overlap between the criteria for metabolic syndrome, including the type and number of symptoms, some substantial differences can also be noted. The differences include: microalbuminuria as a sixth variable for the WHO definition; the diagnostic criteria of these variables; and use of a mandatory variable. Both the WHO and IDF criteria have a mandatory variable; insulin resistance or impaired glycaemic profile for WHO, and central obesity for IDF.

Using a mandatory diagnostic variable has disadvantages as there is the potential that people who are at high-risk of future metabolic disease will be excluded. The IDF criteria has central adiposity as its mandatory variable. Yet obesity is not a feature for all patients with both coronary artery disease and type 2 diabetes (Khan et al., 2010). The WHO definition requires either a known glucose impairment disorder (Table 2), or a diagnosis of insulin resistance in the lowest quartile for the background population taken under hyperinsulinaemic-euglycaemic conditions (World Health Organization, 1999). While diagnosing a glucose impairment disorder is a routine clinical practice, this insulin resistance test is not straight-forward. Briefly, the person is cannulated and

fixed-rate, high-dose insulin is infused (hyperinsulinaemia). Simultaneously, glucose is infused at a variable rate to maintain euglycaemia. Thirty minutes after the last rate change of glucose infusion (normally about three hours after test initiation), measures can be taken to perform the insulin resistance calculation (DeFronzo, Tobin, & Andres, 1979). The high degree of technical expertise to conduct and monitor this test, combined with costs, staff time and other equipment involved makes it clinically impractical and too expensive for routine clinical practice and/or large observational studies.

It can also be argued that using the NCEP ATP III criteria may over-diagnose people who would otherwise be at a lower risk of metabolic disease and this may further burden the medical system. Conversely, lifestyle management is the first line treatment of all of these symptoms that contribute to metabolic syndrome (Grundy et al., 2005). Given that lifestyle management is a benign treatment, if metabolic syndrome is over-diagnosed, there is no risk of harm to the patient. Furthermore, these symptoms can all be managed in primary care by a general practitioner. Regular monitoring may ensure earlier diagnosis of disease progression if necessary and hence access to secondary care.

Understanding the aetiology of metabolic syndrome is further confounded due to the inter-connected nature of many of the symptoms as shown in Figure 3. The directionality of many of the symptom-symptom relationships are not yet fully elucidated, therefore some are currently shown as unidirectional and others bidirectional as according to current knowledge. However, the fact remains that the prevalence of metabolic syndrome is increasing, and prevention is still better than cure. Insulin resistance is identified in the common aetiology of both type 2 diabetes and atherosclerosis (Reaven, 1988). Therefore, understanding why insulin resistance occurs, and/or determining better management strategies may help prevent metabolic syndrome in the future.

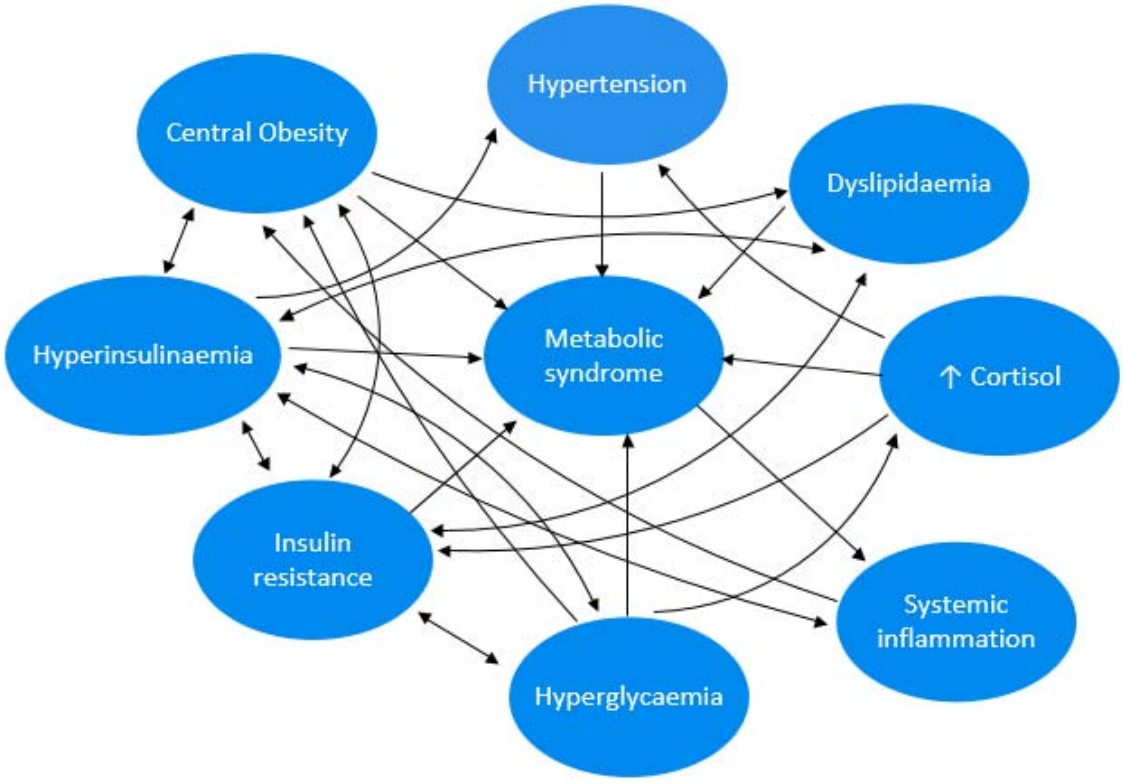

Figure 3: The interconnected nature of the symptoms of metabolic syndrome. Arrows indicate best known evidence of directionality.

Role of insulin

For a complete discussion of the direct risks of chronic hyperinsulinaemia, it is important to review the physiological role of insulin. Insulin's primary role is to assist in the maintenance of blood glucose levels by facilitating the entry of glucose into cardiac muscle, skeletal muscle, adipose and hepatic cells. Insulin also stimulates the liver to store glucose as either glycogen or fatty acids. These fatty acids are converted into low-density and very-low-density lipoproteins. Once exported from the liver, these lipoproteins degrade and the fatty acids become available for use in other tissues, including the adipocytes, which use them to synthesise triglycerides. Once in the adipose tissue, further supplies of insulin inhibit triglyceride breakdown into free fatty acids. With respect to protein, insulin increases the uptake of amino acids into cells and reduces the breakdown of proteins, contributing to its anabolic effects. While insulin can be simplistically described as an "energy storage hormone", it also has several other roles. Insulin affects electrolyte balance by decreasing renal sodium excretion and increasing potassium uptake into other cells.

Table 1: Definitions of metabolic syndrome

	The National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) **3 or more variables**	**World Health Organization (WHO)** **Insulin resistance/Glucose impairment disorder plus 2 or more variables**	**International Diabetes Federation (IDF)** **Central obesity plus 2 or more variables**
Central obesity	Waist circumference > 102 cm (male) > 88 cm (female)	Body mass index > 30kg/m^2 *or* Waist-to-hip ratio > 0.9 (male) > 0.85 (female)	Body mass index > 30kg/m^2 *or* Waist circumference ≥ 90-94 cm (male; ethnicity dependent) ≥ 80 cm (female)
Dyslipidaemia	HDL cholesterol < 1.0 mmol/L (male) < 1.3 mmol/L (female) Triglycerides > 1.7 mmol/L a*nd/or* dyslipidaemic medication	HDL cholesterol < 0.9 mmol/L (male) < 1.0 mmol/L (female) Triglycerides > 1.7 mmol/L a*nd/or* dyslipidaemic medication	HDL cholesterol < 1.03 mmol/L (male) < 1.29 mmol/L (female) Triglycerides > 1.7 mmol/L a*nd/or* dyslipidaemic medication
Glycaemic profile	Confirmed glucose impairment disorder as per Table 2	Confirmed glucose impairment disorder as per Table 2 *or* Normoglycaemia *and g*lucose uptake below the lowest quartile for background population under hyperinsulinaemic, euglycaemic conditions	Fasting glucose ≥ 5.6 mmol/L *or* Confirmed type 2 diabetes
Hypertension	Blood pressure > 135/85 mm Hg *and/or* antihypertensive medication;	Blood pressure > 140/90 mmHg *and/or* antihypertensive medication;	Blood pressure > 135/85 mm Hg *and/or* antihypertensive medication;
Microalbuminuria:	N/A	Albumin excretion > 20 μg/min	N/A

Table 2: WHO diagnostic values for diabetes and other glucose impairment disorders.

	Glucose concentration mmol/L		
	Whole blood		Plasma
	Venous	Capillary	Venous
Normal glucose tolerance	< 5.6	< 5.6	< 6.1
Impaired fasting glucose			
Fasting	5.6 - 6.1	5.6 - 6.1	6.1-7.0
2-hour post 75 g glucose load (if measured)	< 6.7	< 7.8	< 7.8
Impaired glucose tolerance			
Fasting	< 6.1	< 6.1	< 7.0
and	*and*	*and*	*and*
2-hour post 75 g glucose load	≥ 6.7	≥ 7.8	≥ 7.8
Diabetes Mellitus			
Fasting	≥ 6.1	≥ 6.1	≥ 7.0
and/or			
2-hour post 75 g glucose load	≥ 10.0	≥ 11.1	≥ 11.1

Insulin is also believed to affect appetite; the acute, rapid post-prandial increase in serum insulin is believed to have a satiating effect (Flint et al., 2006). Conversely, chronic hyperinsulinaemia is associated with leptin resistance and increased hunger (Lustig, Sen, Soberman, & Velasquez-Mieyer, 2004). It is also plausible that many of the hypothalamic processes that increase hunger decrease non-volitional physical activity (Novak & Levine, 2007). This research is supported by insulin resistance in the central nervous system being associated with the decreased energy homeostasis in rodents via the sympathoadrenal response (Vogt & Brüning, 2013).

Progression from normal insulin physiology to diabetes mellitus

One of the key obstacles to early diagnosis of hyperinsulinaemia and prevention of further pathophysiology is that the change from normal insulin physiology to severe type 2 diabetes, (characterised by complete pancreatic failure and potential for

ketoacidosis) takes place over many years. These changes start with a period of pancreatic compensation (hyperinsulinaemia), followed by decompensation and/or atrophy. During the early period of hyperinsulinaemia compensation, the pancreas increases mass via β-cell hyperplasia and/or hypertrophy (Weir & Bonner-Weir, 2004). As euglycaemia is being maintained within a reasonable time-frame, this degenerating metabolic state is unlikely to be detected. This period may also be characterised by reactive hypoglycaemia with accompanying increases in cortisol secretion, and hepatic glucose production (gluconeogenesis). The time-frame for this compensatory period is unknown but postulated to be at least 8-10 years (Zavaroni et al., 1999). Hyperinsulinaemia and/or changes to insulin response following a glucose load may be the only way to detect this period given the nature of the pancreatic adaptation. During this period, markers of metabolic dysfunction, including glycaemic changes, are likely to be considered normal (Kraft, 2011; Weir & Bonner-Weir, 2004).

A lack of readily diagnosable symptoms means hyperinsulinaemia is a "silent disease" and not detectable until pancreatic compensation cannot be maintained and β-cell mass slowly declines. This is demonstrated by a delay in elevated serum insulin after a glucose challenge. It may also be accompanied by impaired fasting glucose and/or impaired glucose tolerance and may be the period often described as "prediabetes". This period of decompensation can take from months to years to develop into "full" type 2 diabetes as rates of progression to type 2 diabetes can range from 5-11% of people with "pre-diabetes" per year. The lower rates of progression are more likely to occur if there is adherence to lifestyle changes (Weir & Bonner-Weir, 2004). Eventually β-cells decline to a critical point and there is a rapid increase in blood glucose, which may be accompanied by symptoms such as thirst, polyuria or weight-loss. It is postulated that gluco-and/or lipotoxicity are involved with this rapid state of change as both hyperglycaemia and high levels of certain free-fatty acids are known to induce β-cell death (Donath & Shoelson, 2011). Typically, patients, who now have unambiguous type 2 diabetes, maintain sufficient insulin secretion to prevent ketoacidosis and the other symptoms of type 1 diabetes. Some patients will have further β-cell loss and will be truly dependent on exogenous insulin for survival.

Weir and Bonner-Weir (2004) propose that there are five stages of evolution between normal insulin physiology and severe type 2 diabetes (compensation; stable adaptation; unstable early decompensation; stable decompensation; and severe decompensation). Kraft (1975, 2011) and separately Hayashi and colleagues (2013) demonstrated five

different patterns of insulin response to a glucose load. Whether Kraft's or Hayashi and colleagues' patterns correlate with Weir and Bonner-Weir's five stages of evolution are yet to be determined.

The question of whether hyperglycaemia itself causes end-stage organ damage remains unanswered. Apparent improvements in metabolic state may be due to lifestyle changes, including decreased caloric intake, weight-loss and increased physical activity. However, these lifestyle changes may simply allow an individual to maintain glycaemic control, but their hyperinsulinaemic state may remain unchanged. Weir and Bonner-Weir (2004) argue that people can move between compensation and stable decompensation in either direction. Kraft (2011) counter-argues that other metabolic symptoms, such as hypertension, indicate the beginning of "end-stage" diabetic disease, rather than what is conventionally considered early metabolic disease. Further research will be needed to determine if lifestyle changes can cause remission of pancreatic decompensation.

Insulin resistance and impaired insulin homeostasis

Insulin resistance is well-established as underpinning many significant chronic health conditions including type 2 diabetes, metabolic syndrome, cardiovascular disease, some cancers and Alzheimer's disease (Ceriello & Motz, 2004; Pollak, 2008; Weir & Bonner-Weir, 2004; Zavaroni et al., 1999). However, in order to understand insulin resistance, we must first consider the concept of impaired insulin homeostasis. Homeostasis is the maintenance of a bodily system so that internal conditions remain stable. There are two main expressions of impaired insulin homeostasis: hypoinsulinaemia, and hyperinsulinaemia. Hypoinsulinaemia is where the body is either unable to manufacture, or manufactures a significantly lesser amount of insulin than someone with normal insulin homeostasis, for example type 1 diabetes. Hyperinsulinaemia can be considered isolated, such as that associated with an insulinoma, or compensatory. Compensatory hyperinsulinaemia is theorised to occur only in concert with insulin resistance. As insulinomas are relatively rare and not usually associated with insulin resistance, for the purposes of this thesis only compensatory hyperinsulinaemia will be discussed, termed "hyperinsulinaemia" unless emphasis is required.

Insulin resistance

Insulin resistance is defined as "the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilisation in an individual as much as it does in the general population" (Lebovitz, 2000; Shanik et al., 2008). The glucose uptake rate is the rate at which glucose is absorbed by the hepatic or muscle cells and can be affected by four intertwined factors: 1) the quantity of glucose that needs to be removed from the blood stream (restoration of euglycaemia); 2) the availability of glucose transporters, or other ability of the cell to absorb the glucose (the glucose uptake rate); 3) the capacity of the cell to take up glucose (i.e. how "full" is it already) and 4) the rate at which the glucose/glycogen is being eliminated. Under hyperinsulinaemic-euglycaemic conditions, energy output, hepatic gluconeogenesis, and glycogenesis are controlled, therefore, the rate of glucose infusion will equal the glucose uptake rate due to the hyperinsulinaemic state. This forms the basis for the gold-standard test for assessing insulin resistance being the hyperinsulinaemic-euglycaemic clamp test as previously described

The pathology behind insulin resistance is believed to include glucose transporter (GLUT), especially GLUT4 down regulation (Scheepers, Joost, & Schurmann, 2004). The mechanisms for this are still under debate, but may include increased cortisol and glucose or insulin over-stimulus (Flores-Riveros, McLenithan, Ezaki, & Lane, 1993; Scheepers et al., 2004). This insulin resistance may be acute or chronic. For example, acute insulin resistance is posited to occur in times of acute cortisol secretion (stress), starvation, severe carbohydrate restriction, or after consumption of excessive carbohydrate (e.g. a high amount of refined sugar). Acute insulin resistance is believed to be a normal, transient, physiological condition. Once resolved, the normal function of GLUT4 is restored. Conversely, chronically down-regulated GLUT4 would result in persistent insulin resistance. Acute states of insulin resistance are posited to occur as a normal physiological state and are unlikely to be detected. Therefore, further references to insulin resistance refer to the persistent or chronic state.

Using the theory of insulin resistance as the basis for metabolic syndrome research has disadvantages. The gold-standard for measuring is the glucose uptake below the lowest quartile for the background population under investigation using hyperinsulinaemic, euglycaemic conditions (World Health Organization, 1999). There are two significant flaws with this interpretation. Firstly, different glucose uptake rates are noted in different populations. For example, Maori women were noted to have a higher degree

of insulin resistance than European women for the same BMI (McAuley, Williams, Mann, Goulding, & Murphy, 2002). What then is the interpretation of "population"? Should the population be considered "Women" or should the Maori and European populations be considered separate populations? Because of the higher degree of insulin resistance, the Maori women were considered to be at higher risk of developing type 2 diabetes later in life compared to the European cohort (McAuley et al., 2002). If the two populations were considered to be separate populations, the different insulin resistance cut-offs are unlikely to reflect actual disease risk.

The second significant flaw is using the lowest quartile of the population under investigation as this precludes any reference that can be applied between populations as described above. It also means that if insulin resistance becomes more prevalent within the community, this relative reference will also change and will be less likely to indicate risk of future metabolic disease. An absolute reference directly related to risk of future pathologies is required. It may be appropriate to differentiate this reference range by demographic factors such as age, gender, or ethnicity as occurs with other biological markers such as ferritin (Waikato District Health Board, 2015).

Although using insulin resistance as the key concept of metabolic syndrome provides a conceptual framework that allows seemingly unrelated biological phenomena to form a pathological construct (Boehm & Claudi-Boehm, 2005), there is a significant flaw in focusing on insulin resistance. There are few direct mechanistic links between the cell's ability, or lack thereof, to remove glucose from the blood stream and other pathologies. Emerging research suggests fatty acid metabolism may also become resistant to insulin's actions resulting in an increased risk of hypertension (Egan et al., 1996). However, this does not sufficiently explain all the other pathologies associated with insulin resistance.

An important point that may have been overlooked in the early stages of the research is the common aetiology of resistance to insulin-mediated glucose disposal and compensatory hyperinsulinaemia (Reaven, 2002). While there is a plethora of research based on insulin resistance, the compensatory hyperinsulinaemia appears to have been under-recognised and is deserving of further investigation as it is plausible that compensatory hyperinsulinaemia may provide further mechanistic explanations for the pathologies associated with metabolic syndrome.

Hyperinsulinaemia

Definition

There is no precise definition of hyperinsulinaemia. It is often described as "more insulin than normal to achieve euglycaemia"; essentially the same as insulin resistance. Where a reference range is available, it is normally based on fasting levels and include 5-13 μU/mL (Labtests, 2012), ≤ 30 μU/mL (Kraft, 1975), and 18-173 pmol/L (3-28 μU/mL) (Waikato District Health Board, 2015). However, very few studies define a "normal level of insulin" as many studies define hyperinsulinaemia based on quantiles (Laakso, 1993; Lan-Pidhainy & Wolever, 2011; Nilsson, Nilsson, Hedblad, Eriksson, & Berglund, 2003). A few studies have been more specific. Both a fasting serum insulin of ≥12.2 μU/mL in the presence of euglycaemia (McAuley et al., 2001) and a range of 8-11 μU/mL "between meals" and up to 60 μU/mL "after meals" (Iwase, Kobayashi, Nakajima, & Takatori, 2001) have been proposed. There are also practical, methodological issues with determining insulin resistance under the World Health Organization (WHO) conditions that will be discussed later in this review.

Aetiology

The aetiology of hyperinsulinaemia is not yet fully elucidated. Although there are several theories, further research will likely show a multimodal pathology. What can be deduced from physiological principles is:

1. Healthy cells are subjected to acute hyperglycaemia.

2. Although many cells can absorb glucose without using insulin (glucose transporter-1 (GLUT1)) hyperglycaemia causes insulin to be released from pancreatic cells to facilitate absorption, especially in muscle and adipose cells (GLUT4) (Wilcox, 2005)

3. Insulin binds to cellular insulin receptors and facilitates translocation of GLUT4 to the cellular surface. During this process the insulin and its receptor are absorbed into the cell to be replaced from the internal pool of insulin receptors. (Grunberger, Taylor, Dons, & Gorden, 1983).

4. This acute insulin resistance is of no consequence as long as the cell has viable GLUT4 on the cellular surface. However, GLUT4 have a relatively short half-life (Schnurr, Reynolds, Komac, Duffy, & Dunlap, 2015)

5. If hyperglycaemia persists, the pancreas maintains insulin secretion. This may deplete the insulin receptors faster than they can be replaced.

6. During this period where the cells are replacing their insulin receptors, moderately elevated blood glucose levels, (such as that immediately found after a normal meal) may need slightly higher than normal insulin levels to restore normoglycaemia. This moderate hyperinsulinaemia may delay the return to normal insulin receptor function (acute insulin resistance).

7. This state of insulin resistance due to down-regulated insulin receptors is reversible should the person not be subjected to further episodes of hyperglycaemia. It does not matter whether this is via high, but acute, blood glucose elevations, or moderately elevated glucose levels over a prolonged period.

8. Prolonged impaired insulin signalling impedes GLUT4 translocation to the cellular surface thus causing impaired glucose uptake and prolonging hyperglycaemia, causing a positive feedback cycle. This will both aggravate and prolong the insulin resistance, potentially turning it from a transitory state to a persistent or chronic state.

The complexity of the insulin receptor regulation, combined with the availability of glucose transporters and factors that influence insulin secretion mean that it is impossible to generalise whether insulin resistance precedes or follows hyperinsulinaemia. It is more plausible that different individuals have different triggers in the cycle. These triggers may include genetic factors, excessive carbohydrate, corticosteroids (endogenous or exogenous), free fatty acids, leptin, or certain medications; each of these are discussed below.

Fructose

Fructose is hepatically metabolised into ATP and/or triglycerides in a process that is competitive with, and preferential to, glucose. If excessive fructose is consumed, glucose will not be metabolised causing hyperglycaemia and subsequent hyperinsulinaemia. (Farooqui, Farooqui, Panza, & Frisardi, 2012; R. J. Johnson et al., 2009). Excessive fructose also results in hyperuricaemia which is associated with reduced endothelial nitric oxide causing vasoconstriction, endothelial dysfunction and insulin resistance (R. J. Johnson et al., 2009).

Hyperglycaemia

Hyperglycaemia alone can aggravate insulin resistance (Vuorinen-Markkola, Koivisto, & Yki-Jarvinen, 1992). Along with excessive carbohydrate ingestion, other mechanisms for this process include hepatic insulin resistance. Increased plasma

insulin slows hepatic gluconeogenesis but this process can be impaired by hepatic insulin resistance leading to peripheral hyperglycaemia and further insulin secretion (Hundal et al., 2000).

Corticosteroids

It is known that corticosteroids, especially endogenous cortisol, causes down-regulation of GLUT-4 receptors, thus preventing glucose uptake and provoking hyperinsulinaemia in the presence of hyperglycaemia. Long-term courses of exogenous corticosteroids, such as prednisone, are known to cause "drug-induced" type 2 diabetes, which may resolve after the medication is discontinued. Not every patient on long-term corticosteroids will develop drug-induced diabetes. Therefore, it is plausible that the patient's degree of insulin resistance at baseline influences disease development/progression. Given that stress causes a temporary rise in cortisol levels, it is also plausible that prolonged stress may be another cause of hyperinsulinaemia (Björntorp & Rosmond, 1999).

Leptin

Appetite control is mediated from the hypothalamus in response to a balance between leptin and insulin controlling neuropeptide Y expression (Porte, Baskin, & Schwartz, 2002). This balance is believed important to manage caloric intake over longer periods of time when meals can vary in size, frequency and composition. Leptin secretion is slow to change as it is influenced by total body fat mass and total caloric intake, while insulin secretion is highly responsive to food ingestion and will change quickly with every meal. Leptin is also highly influenced by insulin as it is released from fat stores by mechanisms that appear to involve glucose flux (Porte et al., 2002). Experimental evidence shows that reducing insulin secretion reduces leptin resistance, suggesting a relationship between hyperinsulinaemia and hyperleptinaemia (Lustig et al., 2004). It is not yet clear whether hyperleptinaemia is causative of hyperinsulinaemia beyond the association of obesity and an increase in free fatty acids.

Medication-induced

There are a number of medications known or suspected to cause hyperinsulinaemia and/or contribute to insulin resistance. Exogenous corticosteroids (prednisone) and exogenous insulin and insulin secretagogues (sulphonylureas) have had their mechanisms discussed. Other medications include the antipsychotics (e.g. clozapine),

and statins (Taylor, Paton, & Kerwin, 2007). The mechanisms for these medications causing hyperinsulinaemia are currently unknown.

Due to the nature of insulin receptor regulation, it is also plausible that insulin sensitivity of the cells can be restored. This would require the absence of both hyperinsulinaemia and hyperglycaemia. Case studies indicate that a carbohydrate restricted diet may facilitate this effect (Kraft, 1975).

Summary

Overall, it should be recognised that hyperinsulinaemia is independent to insulin resistance: Hyperinsulinaemia is excessive insulin secretion, while insulin resistance is impaired glucose uptake. This review investigates the both the mechanistic and epidemiological evidence that links hyperinsulinaemia to metabolic disease. Although there is good quality research mechanistically linking hyperinsulinaemia to subsequent pathologies, there is a paucity of good epidemiological evidence. Given the intertwined nature between insulin resistance and hyperinsulinaemia as depicted above, it can be assumed that the majority of people with insulin resistance are also hyperinsulinaemic. Therefore, if no epidemiological data was available, this review used epidemiological research based on insulin resistance as a proxy for hyperinsulinaemia.

Direct effects of hyperinsulinaemia

As shown in Table 3, hyperinsulinaemia can be mechanistically and epidemiologically linked to metabolic syndrome, gestational and type 2 diabetes and therefore, cardiovascular and other diseases with an increased prevalence in those with metabolic syndrome (Ceriello & Motz, 2004; Stout, 1990; Weir & Bonner-Weir, 2004; Zavaroni et al., 1999). It is also an independent risk factor for a number of other diverse conditions including diet-induced obesity, osteoarthritis, certain cancers, especially breast and colon/rectum, and Alzheimer's disease and other dementias. (Dankner, Chetrit, Shanik, Raz, & Roth, 2012; Feng et al., 2013; Giovannucci et al., 2010; Mehran et al., 2012; Pollak, 2008; Yan & Li, 2013).

Other conditions that may be associated with hyperinsulinaemia, via either epidemiological evidence or potential mechanism of action, include gout, tinnitus, schizophrenia and autism (Fam, 2002; Kraft, 1998; U. Meyer, Feldon, & Dammann, 2011; Monzo et al., 2013). Further research is needed to confirm these associations.

Table 3: Biological systems and disease states affected by hyperinsulinaemia, and associated mechanisms of action**.**

Biological System	Disease	Mechanism	Direct or indirect mechanism	Mechanism of action	Epidemiology
Cancer*	Cancer (Breast, ovarian, colon, bladder, pancreas, liver)	Increased insulin-like growth factor IGF-1 enhances cellular growth and proliferation.	Direct	(Matafome, Santos-Silva, Sena, & Seiça, 2013; Pollak, 2008)	(Giovannucci et al., 2010)
		Enhanced glucose uptake and utilisation enhances cellular growth and proliferation.	Both	(Giovannucci et al., 2010)	(Giovannucci et al., 2010)
		Increased production of reactive oxidative species causes derangement of DNA and enzymes involved with repair mechanisms (enhanced by hyperglycaemia).	Indirect	(Bayir, 2005; Ceriello & Motz, 2004; Wiseman & Halliwell, 1996)	(Bayir, 2005; Ceriello & Motz, 2004; Wiseman & Halliwell, 1996)
		Increased sex-hormone production and decreased sex hormone binding globulin causes increased cellular growth and proliferation (enhanced by obesity).	Direct	(Giovannucci et al., 2010)	(Giovannucci et al., 2010)
Circulatory	Atherosclerosis	Arterial wall damage caused by inflammation, increased proliferation and migration of arterial smooth muscle cells. Stimulation of the mitogen-activated protein kinase pathway.	Both	(Monnier, Hanefeld, Schnell, Colette, & Owens, 2013; Stout, 1990)	(Donnelly, Emslie-Smith, Gardner, & Morris, 2000; Folsom et al., 1997; Huxley, Barzi, & Woodward, 2006; Stout, 1990)
	Cardiomyopathy	Microvascular disease, including changes to capillary permeability, microaneurysm formation, vasoconstriction and microthrombi.	Both	(Maisch, Alter, & Pankuweit, 2011; Tarquini,	(Maisch et al., 2011; Tarquini et al., 2011)

Biological System	Disease	Mechanism	Direct or indirect mechanism	Mechanism of action	Epidemiology
		Increased myocardial fibrosis by increased reactive oxidative species, deranged collagen production.		Lazzeri, Pala, Rotella, & Gensini, 2011)	
		Diabetic neuropathy causes changes to catecholamines, which further impairs myocardial function.			
	Endothelial dysfunction	Vasoconstriction and pro-atherosclerotic effects from decreased nitric oxide bioavailability and action and increased thromboxane. Enhanced by increased reactive oxidative species and advanced glycation end-products.	Both	(Ceriello & Motz, 2004; Chilelli, Burlina, & Lapolla, 2013; Rask-Madsen & King, 2007)	(Donnelly et al., 2000)
	Thrombosis	Hyperinsulinaemia impairs fibrinolysis while hyperglycaemia causes increased blood coagulability .	Indirect	(Stegenga et al., 2006)	(Donnelly et al., 2000)
Gastrointestinal	Diabetes: Gestational	Pre-existing insulin resistance and increased demand for insulin.	Direct	(Kaaja & Rönnemaa, 2008)	(Kaaja & Rönnemaa, 2008)
	Diabetes: Type 2	Prolonged insulin resistance eventuating in β-cell failure. Down-regulation of GLUT4.	Direct	(Flores-Riveros et al., 1993; Scheepers et al., 2004; Weir & Bonner-Weir, 2004)	(Zavaroni et al., 1999)

Biological System	Disease	Mechanism	Direct or indirect mechanism	Mechanism of action	Epidemiology
Endocrine	Hyper-triglyceridaemia	Increased triglyceride production.	Direct	(Medina-Santillán et al., 2013; Olefsky, Farquhar, & Reaven, 1974)	(Marchesini et al., 1999)
	Non-alcoholic fatty liver disease	Fatty acid production exceeds distribution capacity. Aggravated by inflammation and oxidative stress.	Direct	(Medina-Santillán et al., 2013)	(Marchesini et al., 1999)
	Chronic inflammation	Stimulation of mitogen-activated protein kinase pathway; glycaemic variability; hyperglycaemia and/or obesity influences increased cytokine production.	Indirect	(Matafome et al., 2013; Monnier et al., 2013)	(Marques-Vidal et al., 2013)
	Obesity	Decreased lipolysis.	Direct	(Choi et al., 2010)	(Swinburn et al., 2009)
		Lack of appetite suppression.	Direct	(Lustig et al., 2004; Porte et al., 2002)	(Yu et al., 2013)
Nervous	Alzheimer's disease and vascular dementia	Endothelial dysfunction resulting in microvascular disease, metabolic disturbances and neuronal damage.	Direct	(Ceriello & Motz, 2004; Humpel, 2011; Rask-Madsen & King, 2007)	(Erol, 2008; Feng et al., 2013; Razay & Wilcock, 1994)
		Increased blood coagulability and/or impaired fibrinolysis cause multiple thrombotic events.	Both	(Barkhof, Fox, Bastos-Leite, & Scheltens, 2011; Stegenga et al., 2006)	
		Changed regulation of β-amyloid and tau protein (Alzheimer's disease).	Direct	(Humpel, 2011; Qiu & Folstein, 2006)	
		Decreased synaptic plasticity caused by dysregulated PSA-NCAM** interactions (Alzheimer's disease).	Direct	(Monzo et al., 2013)	

Biological System	Disease	Mechanism	Direct or indirect mechanism	Mechanism of action	Epidemiology
	Peripheral neuropathy	Increased production of reactive oxidative species and advanced glycation end-products enhanced by hyperglycaemia.	Indirect	(Ceriello & Motz, 2004; Chilelli et al., 2013)	(Donnelly et al., 2000; Sadosky et al., 2013)
		Insulin resistance in the dorsal root ganglion neurons.	Both	(Kim, McLean, Philip, & Feldman, 2011)	
	Retinopathy	Hyperglycaemia and endothelial dysfunction contribute blood-retinal barrier breakdown. Aggravated by excess advanced glycation end-products.	Direct	(Chilelli et al., 2013; Donnelly et al., 2000; Poulaki et al., 2002)	(Chilelli et al., 2013; Donnelly et al., 2000; Poulaki et al., 2002)
Skeletal	Osteoporosis	Increased reactive oxidative species and/hyperglycaemia cause collagen breakdown, impairs new collagen synthesis and compromises mensenchymal cells.	Indirect	(Yan & Li, 2013)	(Yan & Li, 2013)
Urinary	Nephropathy	Microvascular disease, including changes to capillary permeability, microaneurysm formation, vasoconstriction and microthrombi.	Direct	(Kang et al., 2002; Rask-Madsen & King, 2007)	(Donnelly et al., 2000; Hamer & El Nahas, 2006)
		Increased production of reactive oxidative species and advanced glycation end-products enhanced by hyperglycaemia.	Indirect	(Chilelli et al., 2013; Forbes, Coughlan, & Cooper, 2008)	

*While cancer is not typically classified as a "biological system", due to its recognition and impact as a key chronic disease, it was decided that it warrants a classification on its own, rather than be integrated into individual biological systems.

**PSA-NCAM = polysialic acid - neural cell adhesion molecule.

Pathophysiological mechanisms

Hyperinsulinaemia affects the body via five main mechanisms: Increased reactive oxidative species and advanced glycation end-products; increased insulin-like growth factor-1 (IGF-1); hyperglycaemia; increased fatty acid/triglyceride production; and by affecting different hormones and cytokines.

Reactive oxidative species

Reactive oxygen species is a collective term that includes both oxygen radicals and non-radical oxidising agents such as hydrogen peroxide (Bayir, 2005). Reactive oxidative species are also produced during, and involved in, many metabolic processes including enzymatic reactions, gene expression and signal transduction (Bayir, 2005). Generally, the actions of intracellular reducing agents such as antioxidants prevent reactive oxidative species-mediated damage. However, a number of factors can contribute to excessive production of reactive oxidative species including excessive calorie consumption and the presence of various pro-inflammatory mediators, including tumour necrosis factor-α (Bayir, 2005). Once produced, reactive oxidative species can interact with numerous cellular components including DNA, lipids, and amino acids. Damage to DNA is likely to be the underlying mechanism for reactive oxidative species being associated with cancer and early aging (Wiseman & Halliwell, 1996). Polyunsaturated fatty acids are considered very susceptible to reactive oxidative species damage, triggering lipid peroxidation, which can affect cell membrane fluidity and integrity, potentially being the mechanism for endothelial damage (Bayir, 2005). Amino acids such as cysteine and methionine are very susceptible to reactive oxidative species damage. Changes to these amino acids are implicated in the development of Alzheimer's disease (Eto, Asada, Arima, Makifuchi, & Kimura, 2002).

Hyperinsulinaemia is associated with increased reactive oxidative species, although the exact mechanism is disputed. Hyperinsulinaemia is mechanistically linked to excessive serum glucose and free fatty acids. Either substrate can cause increased reactive oxidative species production (Ceriello & Motz, 2004). Insulin has also been demonstrated to have some inhibitory effects on reactive oxidative species production that may be independent of its effects on glycaemia (Monnier et al., 2013). However, reducing insulin-stimulated nutrient uptake into the cell is also believed to decrease reactive oxidative species production (Ceriello & Motz, 2004). Further research is required to better understand these mechanisms.

Over-nutrition is also thought to be responsible for the formation of advanced glycation end-products via non-enzymatic glycation and glyco-oxidation processes (Chilelli et al., 2013). Defective renal excretion of advanced glycation end-products, as seen with diabetic nephropathy, and consumption of exogenous advanced glycation end-products increases advanced glycation end-product plasma levels. Advanced glycation end-products are believed to contribute to changes in the microvascular systems and also promote changes to inflammatory, oxidative and other degenerative processes of various chronic diseases including neuropathies (Chilelli et al., 2013).

Growth factors (IGF, vascular endothelial growth factor)

Insulin, IGF-1 and other substances such as vascular endothelial growth factor (VEGF) can stimulate the growth and division of many cells. Insulin can mediate cellular division but may also stimulate cancer cell proliferation and metastasis (Giovannucci et al., 2010). Most importantly, insulin increases the bioavailability of IGF-1, thus insulin is indirectly implicated in all IGF-1 mediated processes. These processes include changes to vascular structures, increases to cellular division and prevention of apoptosis.

Hyperglycaemia

Hyperglycaemia commonly follows hyperinsulinaemia (Weir & Bonner-Weir, 2004) but there is little information to suggest whether fasting glucose, peak glucose, or area-under-the curve (AUC) have the most adverse health impact. Cancer cells have a continuously high glucose uptake, which enhances cellular growth and proliferation (Giovannucci et al., 2010); hyperglycaemia augments this process. Hyperglycaemia allows IGF-1 to stimulate vascular smooth muscle proliferation, which is a hall-mark of both cancer and atherosclerosis. Blood coagulability is also increased by hyperglycaemia irrespective of insulin levels (Stegenga et al., 2006).

Increased fatty acid and triglyceride production

Hyperinsulinaemia influences both free fatty acid and triglyceride production (Olefsky et al., 1974). While the processes that occur during hepatic de novo lipogenesis are not disputed, there is debate as to whether hyperinsulinaemia precedes, or are a consequence of, fatty liver (Vanni et al., 2010). Nevertheless, elevated triglyceride levels are recognised to be a key component of metabolic syndrome (Table 1) while fatty liver may be considered a hepatic manifestation of metabolic syndrome and may progress to cirrhosis or hepatocellular cancer (Vanni et al., 2010). Elevated triglyceride

levels may also further exacerbate leptin resistance (Banks et al., 2004; Farooqui et al., 2012).

Hormone / cytokine production (sex hormones, inflammation, obesity)

Hyperinsulinaemia is involved with adiposity via increased appetite and triglyceride production, thereby increasing adiposity (Bugianesi, McCullough, & Marchesini, 2005; Folsom et al., 1997). Adipose tissue is now well-established as an endocrine organ and produces both hormones and cytokines that are used for cellular communication. Hypertrophic adipose tissues activate inflammatory and stress pathways and decreases insulin response. This results in increased cytokine production including TNF-α, vascular endothelial growth factor and leptin, while adiponectin expression is decreased (Matafome et al., 2013). These actions contribute to decreased glucose and lipid uptake, leading to further reductions to adiponectin secretion and adipogenesis as well as contributing to further insulin resistance. Decreased glucose uptake means there is less glycerol within the adipocyte to esterify free fatty acids, allowing them to infiltrate and accumulate in other tissues.

Adiponectin decreases proliferation of cell types including adipocytes, endothelial cells and cancer cells (Matafome et al., 2013). The role of leptin is yet to be fully understood, but it is accepted that hyperinsulinaemia and hyperleptinaemia results in central leptin resistance, and consequent prevention of appetite suppression and promotion of further obesity (Lustig et al., 2004; Martin, Qasim, & Reilly, 2008; Porte et al., 2002). Hyperleptinaemia is also linked to increased inflammatory cytokines, changes in nitric oxide, and further endothelial injury (Martin et al., 2008).

Hyperinsulinaemia is also believed to elevate plasminogen activator inhibitor type-1 (PAL-1) levels, with associated impaired fibrinolysis and increased risk of thrombosis. When combined with the increased coagulation from hyperglycaemia, this may explain why over 80% of people with type 2 diabetes have a thrombotic death (Stegenga et al., 2006).

Diagnosis

Diagnosing hyperinsulinaemia is challenging partly because the health effects of insulin resistance and hyperinsulinaemia have been conflated. Further challenges arise when interpreting the available literature. As previously discussed on page 23, fasting insulin levels have been assessed as a means of diagnosing hyperinsulinaemia with differing

results. But it is not just the insulin level alone that is problematic. How and when sampling occurs will also cause variation to results. Insulin levels are higher in serum compared to plasma samples meaning that studies reporting serum insulin cannot be compared directly to plasma insulin (Feldman & Chapman, 1973; Henderson, 1970). Insulin secretion is pulsatile leading to significant levels in plasma insulin in a short space of time. It is recommended that the mean of three samples taken at five minute intervals be used if a fasting insulin level is required (Wallace, Levy, & Matthews, 2004), however this rarely seems to happen in practice. Single fasting insulin samples can have a coefficient of variation of 25-50% (Mather et al., 2001). This variation decreases testing sensitivity and is perhaps why fasting insulin is not recommended to be used clinically (Samaras et al., 2006).

It is unknown whether insulin resistance testing can be used to diagnose hyperinsulinaemia. As previously discussed on page 21, the gold standard for measuring insulin resistance is the hyperinsulinaemic-euglycaemic clamp test. The lowest quartile of glucose uptake rate defines insulin resistance for that study population. Figures for this lower quartile have ranged from < 4.7mg/kg ·min to ≤ 6.3M·mU^{-1}·L^{-1}, however differences in insulin infusion rates, glucose disposal rate calculations, and background populations under investigation means that there are limits to the generalisability of these results (Bergman, Ider, Bowden, & Cobelli, 1979; Diamond, Thornton, Connolly-Diamond, Sherwin, & DeFronzo, 1995; Mari, Pacini, Murphy, Ludvik, & Nolan, 2001; McAuley et al., 2001; Tam et al., 2012). Furthermore, given the complexity of the procedure, the hyperinsulinaemic-euglycaemic clamp test has little to no clinical application (McAuley et al., 2001).

A further complication to using the clamp test to assess hyperinsulinaemia is that the high dose infusion of insulin will confound any effects of endogenous insulin secretion. As theorised above, the damage associated with hyperinsulinaemia is due to the continuous action of insulin in the tissues. The amount of insulin normally present in the tissues cannot be measured during the clamp process. It is unknown whether glucose uptake rates correlate with insulin secretion.

A number of tests have been developed that are validated against the hyperinsulinaemic-euglycaemic clamp that have more clinical applicability. Those based on fasting insulin include homeostatic model assessment (HOMA or HOMA2), McAuley Index, and the quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000; Mari et al.,

2001; McAuley et al., 2001). Although HOMA has since been refined to the HOMA2 model, both are modelled on the combination of fasting insulin to fasting glucose. The original HOMA has an 89% sensitivity and 67% specificity compared to hyperinsulinaemia-euglycaemic clamp (Tam et al., 2012). The McAuley index is calculated from fasting insulin and fasting triglyceride levels with 61% sensitivity and 85% specificity (McAuley et al., 2001).

Another insulin resistance test, the oral glucose sensitivity index (OGIS), is modelled on the results derived from an oral glucose tolerance test (Mari et al., 2001). OGIS uses both blood insulin and glucose levels at baseline, 120 min and 180 min. A spreadsheet is recommended for the calculations (available from http://webmet.pd.cnr.it/ogis/download.php). The OGIS is validated against the hyperinsulinaemic-euglycaemic clamp assessments for insulin resistance, but as previously stated, the generalisability of clamps is limited.

Both the OGIS and tests based on fasting insulin levels have more clinical applicability for assessing insulin resistance compared to the hyperinsulinaemic-euglycaemic clamp test. However, insulin resistance testing has never translated to improvements in disease risk calculations. The WHO definition for insulin resistance means that one in four people would be diagnosed with insulin resistance; a figure that may be unrelated to their actual health risks (World Health Organization, 1999). Analysis from the Women's Health Initiative Biomarkers study showed that although HOMA-IR had a positive association with cardiovascular risk, this became non-significant after adjusting for other risk factors such as HDL-cholesterol (Schmiegelow et al., 2015). There is an argument that HOMA-IR should be used in combination with HOMA-%B for assessing insulin resistance (Wallace et al., 2004).

Emerging research now suggests that insulin response patterns following an oral glucose load may determine hyperinsulinaemic status. Kraft (1975, 2011) demonstrated the variability of insulin response to a 100 g glucose load over 3-5 hours, especially with respect to timing and magnitude of the insulin peak and rate of response decline. Five main insulin response patterns are clearly identifiable; with pattern I being considered normal insulin tolerance. From this research Kraft concluded that the most accurate means of assessing hyperinsulinaemia was a 3-hour oral glucose tolerance test with insulin levels assessed at baseline, 30, 60, 120, and, at minimum, 180 minutes but

240 and 300 minute insulin levels could also be considered. This study was cross-sectional and there are no long-term outcomes.

Hayashi and colleagues (2013) have shown that the insulinaemic pattern produced from sampling every 30 minutes during a 2-hour OGTT can predict the development of type 2 diabetes. An insulin peak delayed beyond 60 minutes being associated with poorer health is common to both Kraft and Hayashi patterns. Further research is required to understand how to apply these patterns to clinical practice.

Collectively these studies show that there is a paucity of research for diagnosing hyperinsulinaemia. Most studies focus on insulin resistance testing, but it remains unknown whether insulin resistance correlates with insulin secretion.

Summary

This review clearly demonstrates that not only is hyperinsulinaemia involved with the aetiology of all of the symptoms of metabolic syndrome, it is also implicated in many other conditions; some of which have previously been considered to be idiopathic, such as tinnitus. This raises many questions with both clinical and research implications. Firstly, what is the prevalence of hyperinsulinaemia? Given its association with metabolic syndrome and fatty liver disease, this warrants investigation. Could early detection and careful management of hyperinsulinaemia decrease the need for medical interventions later in life? Would managing hyperinsulinaemia improve both quantity and quality of life? Yet there are currently too many questions regarding diagnosis. Ensuring a reliable and repeatable result when sampling insulin is challenging. There is no agreed upon reference range, and there are only associations between quantiles and ongoing disease risk. Insulin response patterning may answer some of these questions, but patterning requires more resources than a fasting level. Given the global concerns about the ‘epidemic’ of metabolic diseases, this research needs to be urgently addressed.

Chapter 3: Identifying hyperinsulinaemia in the absence of impaired glucose tolerance: An examination of the Kraft database.

Preface

The previous chapters demonstrate that insulin resistance and hyperinsulinaemia are associated with many different metabolic diseases however, only hyperinsulinaemia can mechanistically explain the resultant pathophysiologies. It has been previously suggested that people with insulin resistance may be detected by taking a careful medical history as those with hypertension, central adiposity or dyslipidaemia can be deemed insulin resistant, and therefore hyperinsulinaemic. But what remains unknown is the directionality of hyperinsulinaemia and these metabolic symptoms but also whether hyperinsulinaemia precedes or follows these other conditions. Furthermore, what remains unknown is what proportion of the population are affected by hyperinsulinaemia. The purpose of this chapter is to investigate what proportion of people might be affected by hyperinsulinaemia, with a special emphasis on people with normal glucose tolerance. This, novel, research will contribute substantially by furthering our understanding of the directionality of hyperinsulinaemia and metabolic disease. This chapter is adapted from a paper submitted to Diabetes Research and Clinical Practice.

Abstract

Objective: Hyperinsulinaemia is associated with development of chronic metabolic disease and is emerging as a health risk independent to that of insulin resistance. However, little is known to what extent hyperinsulinaemia occurs with normal glucose tolerance in lean subjects.

Method: Oral glucose tolerance tests with concurrent insulin assay were conducted during the 1970s-1990s. Participants were classified according to glucose tolerance and insulin response pattern. Analysis of variance compared differences in plasma glucose, plasma insulin, and demographic and metabolic risk factors between groups.

Results: Participants with normal glucose tolerance comprised 54% (n= 4185) of the total cohort. Of these, just over half (n = 2079) showed hyperinsulinaemia despite normal glucose clearance. Obesity had a modest association with hyperinsulinaemia in people with normal glucose tolerance. Fasting insulin had limited value in diagnosing hyperinsulinaemia. The majority of participants (93%) with impaired glucose tolerance or diabetes had concurrent hyperinsulinaemia.

Conclusion: Hyperinsulinaemia in the absence of impaired glucose tolerance may provide the earliest detection for metabolic disease risk and likely occurs in a substantial proportion of an otherwise healthy population. Dynamic insulin patterning may produce more meaningful and potentially helpful diagnoses. Further research is needed to investigate clinically useful hyperinsulinaemia screening tools.

Keywords: Hyperinsulinaemia, insulin, diabetes, oral glucose tolerance test

Introduction

Insulin resistance underpins many chronic non-communicable diseases including cardiovascular disease and type 2 diabetes. However, quantifying insulin resistance has failed to translate to clinical benefit, possibly because of the complexity of measurements. Insulin resistance *per se* cannot explain the associated pathologies, including hypertriglyceridaemia, hypertension and vascular disease. However, a key feature of insulin resistance, especially in an individual with euglycaemia is compensatory hyperinsulinaemia. Emerging research suggests that this chronic compensatory hyperinsulinaemia may be an important and an under-recognised pathology that is independent to insulin resistance (Kelly et al., 2014). Hyperinsulinaemia contributes a common pathway to the aetiology of many non-communicable diseases including cardiovascular disease type 2 diabetes, cancer and dementias (Giovannucci et al., 2010; Maher & Schubert, 2009; Stout, 1990). This may be via mechanisms such as arterial wall damage, microthrombi and vasoconstriction (Rask-Madsen & King, 2007); enhancing cellular growth and proliferation, increasing the risk of deranged DNA (Bayir, 2005; Pollak, 2008); or changed regulation of β-amyloid and tau protein and decreased synaptic plasticity (Monzo et al., 2013; Qiu & Folstein, 2006).

Hyperinsulinaemia is becoming recognised as being one of the earliest symptoms of metabolic disease. For example, elevated fasting insulin occurs up to 24 years prior to the onset of hyperglycaemia and is also posited to precede obesity (Dankner, Chetrit, Shanik, Raz, & Roth, 2009; Ludwig & Friedman, 2014; Mehran et al., 2012). There are clear, direct links (biological and epidemiological) between hyperinsulinaemia and hypertriglyceridaemia and non-alcoholic fatty liver disease (Medina-Santillán et al., 2013). This means that we need to broaden our understanding of hyperinsulinaemia independent to insulin resistance as an early metabolic risk factor.

Currently, hyperinsulinaemia is not clinically used for diagnosing or monitoring metabolic risk as we do not have a clinically reliable reference interval from an easy to implement measure. Fasting insulin levels have a wide coefficient of variation and are unreliable for predicting individual disease risk (Samaras et al., 2006; Widjaja et al., 1999). It is also unknown whether other measures of insulin resistance can accurately predict compensatory hyperinsulinaemia. We also have very little understanding of the extent to which hyperinsulinaemia affects people with differing degrees of glucose

tolerance, especially in people with normal glucose tolerance. For example, we do not know the extent, in populations, at which hyperinsulinaemia occurs in the absence of impaired glucose homeostasis.

During the early 1970s to mid-1990s Dr J.R. Kraft pioneered some of this work. Dr Kraft collected oral glucose tolerance test data with concurrent insulin assay from more than 10,000 individuals (Kraft, 1975). The participants were able to be classified into one of five insulin patterns ranging from normal insulin response (Kraft I) through to hyperinsulinaemic responses (Kraft II-IV) and a hypoinsulinaemic response (Kraft V). However, Kraft's work has a number of limitations. His peer-reviewed paper in 1975 described the algorithm that defined insulin patterns, but the glucose response was described in the archaic Wilkerson points system (Kraft, 1975). This algorithm was also unable to ascertain the pattern if the fasting insulin ranged between 31-50 μU/mL. Kraft proposed a second algorithm to define the insulin patterns in a lay publication (Kraft, 2011). While this algorithm did not exclude any results, the degree of similarity or difference between the two patterns has not been examined. Neither have analyses of Kraft's insulin patterns focussed on people with normal glucose tolerance, nor examined insulin response in relation to demographic, or other risk, factors including (BMI).

This study will explore the incidence of hyperinsulinaemia in the presence of both impaired and normal glucose metabolism by re-analysis of Kraft's original database using a modern perspective, including the WHO definitions of glucose tolerance. It aims to understand the relationship of hyperinsulinaemia to age, gender or BMI in the presence of normal glucose tolerance.

Subjects and methods

Subjects

15,000 patients and healthy volunteers were referred for an oral glucose tolerance test at St Joseph Hospital, Chicago. IL. U.S.A. between 1972 and 1992. St Joseph Hospital is a large, non-profit, teaching hospital based near downtown Chicago. Data collected included plasma glucose, plasma insulin, age, gender, height, and weight.

Reanalysis inclusion:

From this database, we included 3953 men aged older than 20 years, and 3802 women aged greater than 45 years who also had age, height and weight recorded; a total of 7755 participants (Table 4).

Table 4: Participant characteristics

	Total	Men	Women	p	Cohen's d
n	7755	3953	3802		
Diabetes mellitus	1666 (21%)	820 (20%)	846 (22%)		
Impaired glucose tolerance	1762 (23%)	895 (23%)	867 (24%)		
Impaired fasting glucose	142 (2%)	77 (2%)	65 (2%)		
Normal glucose tolerance	4185 (54%)	2161 (55%)	2024 (52%)		
Age (years)	55.2 (14.0)	50 (15.4)	60.6 (9.9)	<0.001	0.75
BMI (kg/m^2)	26.9 (5.2)	26.7 (4.5)	27.0 (5.9)	0.044	0.02
Glucose 0 min (mg/dL)	98 (34)	98 (34)	98 (35)	0.443	-----
Glucose 30 min (mg/dL)	172 (54)	172 (46)	174 (50)	0.027	0.08
Glucose 60 min (mg/dL)	190 (78)	190 (76)	190 (80)	0.781	-----
Glucose 120 min (mg/dL)	157 (92)	155 (90)	159 (94)	0.03	0.07
Glucose 180 min (mg/dL)	120 (85)	112 (80)	127 (88)	<0.001	0.20
Insulin 0 min (μU/mL)	15 (19)	16 (22)	15 (16)	0.018	0.06
Insulin 30 min (μU/mL)	74 (57)	73 (57)	76 (58)	0.035	0.05
Insulin 60 min (μU/mL)	105 (74)	103 (73)	106 (75)	0.040	0.05
Insulin 120 min (μU/mL)	103 (81)	100 (79)	107 (83)	<0.001	0.09
Insulin 180 min (μU/mL)	61 (63)	55 (68)	68 (70)	<0.001	0.21
$AUC_{glucose}$ (mg.hr/dL)	471 (212)	464 (205)	477 (219)	0.006	0.06
$AUC_{insulin}$ (μU.hr/mL)	253 (169)	245 (164)	262 (174)	<0.001	0.11
Glucose 120 min – glucose 0 min (mg/dL)	59 (69)	56 (68)	62 (70)	0.001	0.07

Frequency data are reported as n (%), otherwise mean (SD).
Effect sizes for Cohen's d are interpreted as: Large > 0.5, moderate 0.3-0.5, small ≤ 0.2

Reanalysis exclusion:

Exclusion criteria included a BMI ≤ 17.9 kg/m^2 due to the potential confounder of concurrent illness. Women aged between 20-45 years were excluded due to the potential confounder of pregnancy.

Materials and Methods

Study Protocol

Subjects fasted overnight for 10-16 hours. A fasting venous blood sample was taken, followed by ingestion of 100 g of glucose solution (Glucola, Miles/Ames, Elkhardt, IN.). Subsequent venous samples were collected at 30 minutes, 60 minutes, and each successive hour for between three and five hours as determined by the patient's physician. The blood specimens were measured for glucose and insulin. Originally the ferricyanide method (Autoanalyzer, Technicon Corporation) was used to analyse glucose, but this was later changed to plasma glucose oxidase method (Autoanalyzer, Technicon Corporation, Tarrytown, N.J., Vitros, Johnson and Johnson Clinical Diagnostics, Inc., Rochester, N.Y.). Precision was not reported for either glucose analysis; however, other studies using these methods reported a within-run precision for ferricyanide (CV < 5%) (Passey, Gillum, Fuller, Urry, & Giles, 1977), and plasma glucose oxidase (CV < 3%) (Purcell, Behenna, & Walsh, 1979). Glucose samples analysed with the ferricyanide method were adjusted downward by 10 mg/dL to account for the systematic error, according to the methods of Passey and colleagues (1977).

Plasma insulin was determined from the samples stored at -70°C by the Phadebus Insulin Test, (Pharmacia insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden). Precision was reported as SD = 5 µU/mL up to 150 µU ml (Kraft, 1975).

Ethics

Data re-analysis was granted ethical approval by Health and Disability Ethics Committee (New Zealand) on 30 October 2013. Approval reference: 13/CEN/166 (Appendix B). AUTEC reference: 13/337 (Appendix C).

Analysis

Participant classification

Glucose tolerance

Glucose tolerance was defined using WHO criteria (World Health Organization, 1999). There is no consensus for defining hyperinsulinaemia. Previous research generally classifies participants into groups based on quantiles derived from fasting insulin levels. Recommendations for normal fasting insulin range from 2 µU/mL to 30 µU/mL (Ghani et al., 2014; Kraft, 1974; Laakso, 1993; McAuley et al., 2001). However, earlier

research suggested that fasting insulin levels had no relationship to subsequent insulin response pattern, especially $AUC_{insulin}$, and vice versa (Kraft, 1975). Because of this, we believed that a dynamic pattern would best define normal insulin homeostasis. Using the principles of glucose homeostasis, where glucose returns to near fasting levels in healthy people after two hours, this study continues to define normal insulin metabolism as Kraft I. As insulin secretion first increases, then decreases as β-cell dysfunction progresses towards diabetes (Weir & Bonner-Weir, 2004), we further define normal insulin metabolism occurring only in the presence of normal glucose tolerance.

Insulin tolerance

Insulin tolerance was defined using Kraft patterns. Table 5 shows the original algorithm for determining the 1975 Kraft patterns. Use of a classification tree (Appendix D) determined this algorithm to be overly complex and failed to accurately classify any participant with a fasting insulin between 31 and 49 μU/mL inclusive (n= 440).

Conversely, while the 2008 algorithm (Table 6 and depicted in Appendix E) captured every participant, it was deemed to be overly simplistic as there was little difference for many cases between a "normal" insulin pattern (Kraft I) and a "severely hyperinsulinaemic" pattern (Kraft IV) when the insulin response curves were plotted. These algorithms were combined, along with additional information, such as that from Hayashi and colleagues (2013), and using a chronological classification (Appendix F) to form the 2014 algorithm as outlined in Table 7 and depicted in Figure 4 (glucose responses are from people with normal glucose tolerance only).

A hypoinsulinaemic response (Kraft V) either indicated pancreatic gland dysfunction as shown by an elevated glucose response or assumed to be due to a "low carbohydrate diet" (Kraft, 1975, p. 22). If the latter, then the test was repeated after two weeks of a "high carbohydrate diet", which resulted in a Kraft I –IV pattern. Therefore, participants with Kraft V pattern were excluded from sub-analyses on people with normal glucose tolerance on the assumption that they had a repeated test; the results of which were included in Kraft patterns I-IV.

Table 5: Kraft patterning (1975) algorithm (Kraft, 1975)

Pattern	Description
Kraft I	Normal fasting range 0-30 µU/mL
Normal	½-h or 1-h peak above fasting range
	2nd hour less than 50 µU/mL
	3rd hour less than 2nd hour
	2-h + 3-h sum = < 60 µU/mL
	Subsequent hour values at fasting range (0-30 µU/mL)
Kraft II	Normal fasting range 0-30 µU/mL
Normal peak delayed return	½-h or 1-h peak above fasting range
	2-h + 3-h sum = 60-99 µU/mL (borderline)
	2-h + 3-h sum ≥ 100 µU/mL (abnormal)
Kraft IIIA	Normal fasting range 0-30 µU/mL
Delayed peak	Delayed peak 2-h
Kraft IIIB	Normal fasting range 0-30 µU/mL
Delayed peak	Delayed peak 3-h
Kraft IV	Fasting insulin > 50 µU/mL
Kraft V	All values within the range 0-30 µU/mL

Table 6: Kraft patterning (2008) algorithm (Kraft, 2011)

Pattern	Description
Kraft I	Normal fasting range 0-30 µU/mL ½-h or 1-h peak above fasting range 2-h + 3-h sum = < 60 µU/mL
Kraft II	Normal fasting range 0-30 µU/mL ½-h or 1-h peak above fasting range Delayed return to fasting range 2-h + 3-h sum = > 60 µU/mL
Kraft III	Normal fasting range 0-30 µU/mL Delayed peak 2-h or 3-h Delayed return to fasting range
Kraft IV	Fasting insulin > 30 µU/mL Delayed peak 1-h or 2-h Delayed return to fasting range
Kraft V	All values within the range 0-30 µU/mL

Table 7: Kraft pattern criteria 2014

Kraft Pattern	Description
Pattern I Normal insulin	• Fasting insulin ≤ 30 μU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum < 60 μU/mL
Pattern IIA Borderline	• Fasting insulin ≤ 50 μU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum ≥ 60, < 100 μU/mL OR • Fasting insulin 31-50 μU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum < 60 μU/mL
Pattern IIB Hyperinsulinaemia	• Fasting insulin ≤ 50 μU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum ≥ 100 μU/mL
Pattern III Hyperinsulinaemia	• Fasting insulin ≤ 50 μU/mL • Delayed peak (2-hour or 3-hour)
Pattern IV Hyperinsulinaemia	• Fasting insulin > 50 μU/mL
Pattern V Hypoinsulinaemia	• All values ≤ 30 μU/mL

Calculations and statistical analysis

Area under the curve calculations were performed using the trapezoidal rule. Statistical analysis was performed using Microsoft Excel 2010 or IBM SPSS Statistics 22. Two group comparisons were done using independent t-tests. Comparisons between more than two groups were done with one-way analysis of variance. When the omnibus F-test was significant, post-hoc analysis were used to effect pair-wise comparisons using either normal glucose tolerance or Kraft I pattern as the reference. Sidak-Bonferroni's test was used when equal variance was assumed (Leven's test > 0.5) or Dunnett's T3 when equal variance was not assumed. Statistical significance was set at $p < 0.05$, two-tailed tests were used throughout. The standardised difference between the means was calculated by Cohen's d. Effect size references were defined as: Large > 0.5, moderate 0.3-0.5, small ≤ 0.2.

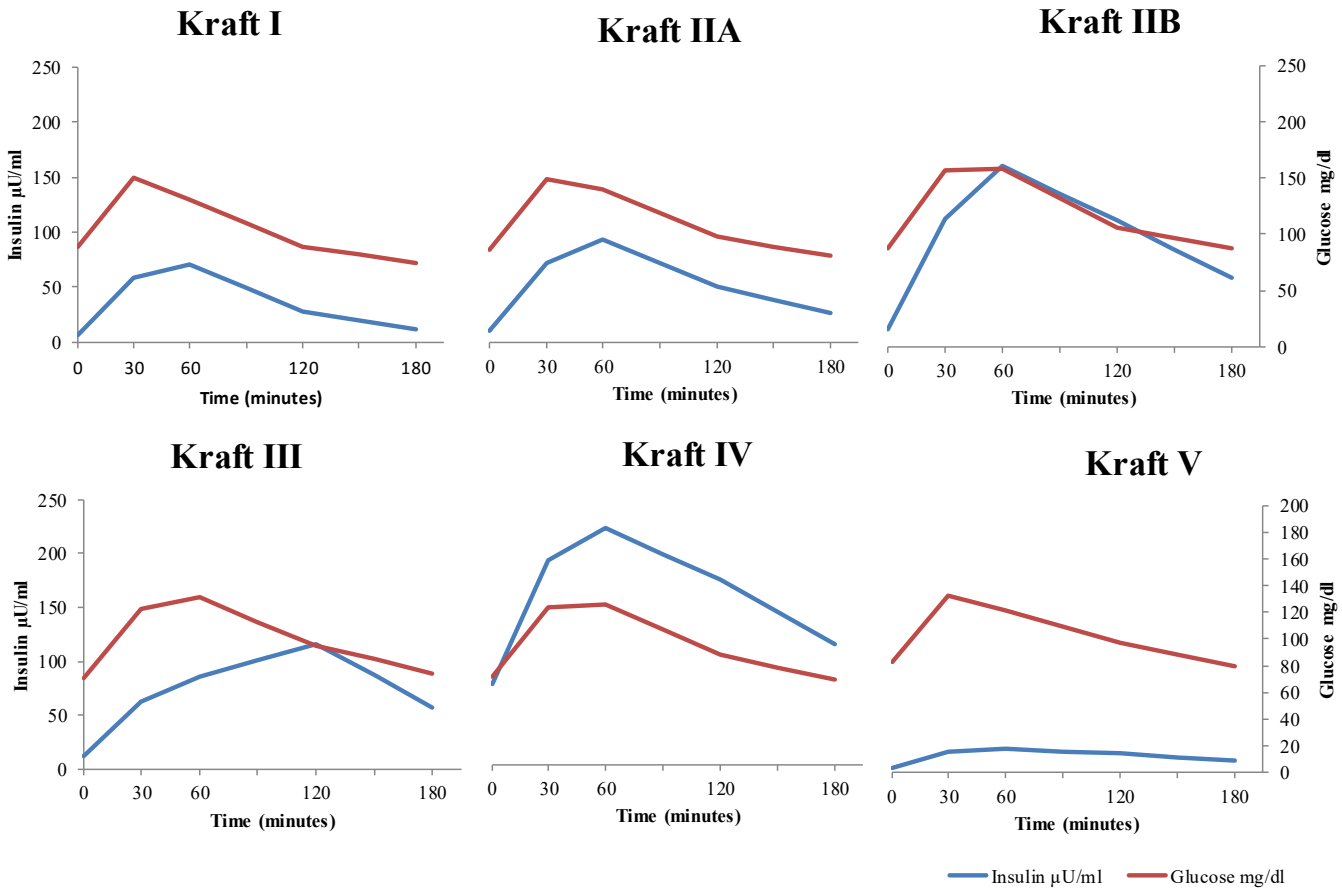

Figure 4: Kraft patterns with glucose response in people with normal glucose tolerance. Data truncated at 180 minutes as not all tests were completed to 300 minutes.

Results

Hyperinsulinaemia and impaired glucose metabolism

These results demonstrate that people with impaired glucose metabolism, overall, have higher insulin levels when compared to people with normal glucose metabolism. Analysis of variance identified significant mean differences between people with normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance and diabetes for fasting insulin (13, 17, 16, and 21 μU/mL respectively, $p < 0.001$) and 2-hr insulin (77, 78, 145, and 128 μU/mL $p < 0.001$) (Table 8). There was a significant difference in $AUC_{insulin}$ analysis across groups: normal glucose tolerance; impaired fasting glucose; impaired glucose tolerance; and diabetes (216, 229, 317, and 281 μU.hr/mL, $p = <0.001$). The majority of participants with either diabetes mellitus (90%) or impaired glucose tolerance (96%) had a hyperinsulinaemic pattern (Kraft IIA, IIB, III, or IV) (Table 8).

Table 8: Diabetes classification by Kraft pattern

	Kraft I	Kraft IIA	Kraft IIB	Kraft III	Kraft IV	Kraft V	Total
Normal glucose tolerance	990 (24%)	961 (23%)	1208 (29%)	807 (19%)	64 (2%)	155 (3%)	4185
Impaired fasting glucose	34 (24%)	22 (15%)	46 (32%)	32 (23%)	6 (4%)	2 (2%)	142
Impaired glucose tolerance	44 (2%)	94 (5%)	389 (22%)	1170 (67%)	44 (2%)	21 (1%)	1762
Diabetes mellitus	32 (2%)	54 (3%)	120 (7%)	1237 (75%)	86 (5%)	137 (8%)	1666
Total	1100 (14%)	1131 (15%)	1763 (23%)	3246 (41%)	200 (3%)	315 (4%)	7755

Hyperinsulinaemia and normal glucose tolerance

From Table 9 it can be noted that only 24% of participants with a normal glucose pattern had a Kraft I pattern. In other words, the majority of people presenting with normal glucose tolerance also demonstrated elevated insulin commensurate with hypersecreting insulin. Using Kraft I participants as a reference, mean BMI increased within participants with Kraft II-IV patterns. The increase was statistically significant ($p < 0.001$), but only had a modest effect size. Although there is a mean age difference between the genders, reflecting the respective cohorts, there was no clinical difference between the genders for mean BMI (male = 26.7 kg/m^2, women = 27.0 kg/m^2). The Kraft pattern could not be determined for most people based solely on their fasting

insulin (Table 9) as there was no clinically meaningful difference for mean insulin between Kraft patterns I-III at baseline. Both the difference between plasma glucose at 120 min and fasting glucose and the $AUC_{glucose}$ showed that although these cohorts all had normal glucose tolerance, those with hyperinsulinaemia, had greater $AUC_{glucose}$ and a longer delay in plasma glucose returning to baseline ($p < 0.001$). All patterns showed a large effect size (Cohen $d > 0.5$), suggesting clinical significance, with the exception of Kraft IIA, which had a moderate effect size.

Discussion

This study examined the presence of hyperinsulinaemia in a large cohort of healthy volunteers and people suspected of having impaired glucose homeostasis, using the previously defined Kraft I pattern as the definition of normal insulin tolerance. These results show that, overall, hyperinsulinaemia affected more than 80% of the study population. This included > 90% of participants with diabetes or impaired glucose tolerance and nearly 75% of people with normal glucose tolerance.

This study is unique in that it features a study design that focuses purely on the analysis of the results from approximately 20 years of medical data that was collected in accordance with the best medical practice of the time. The complete database reflected a population sampling of Chicago I.L with no preselection as to age, gender or ethnicity. The large sample size and the extended time period over which the data were collected reinforce the value of this study. The lack of ethnicity information, co-morbidities, other metabolic information, or long-term outcomes are a limitation to the study. It is also unknown what proportion of people were referred for the test for clinical reasons or as healthy volunteers. Nonetheless, since this information was not collected, they may be considered study delimitations and should not detract from our principal findings. A further potential limitation was that we were unable to differentiate between people with type 1 and type 2 diabetes based on plasma glucose levels. However, a key feature of type 1 diabetes is a hypoinsulinaemic response to a glucose load and likely to be depicted as a Kraft pattern V or, more rarely, pattern I. People with type 1 diabetes are also believed to contribute to a minority of cases of diabetes mellitus and this may be reflected with Kraft pattern V cases comprising about 8% of the cases of diabetes.

Table 9: Participant characteristics: Normal glucose tolerance

	Kraft I	Kraft IIA	Kraft IIB	Kraft III	Kraft IV	Total
n (%)	990 (24)	961 (24)	1208 (30)	807 (20)	64 (2)	4030
Female sex (%)	402 (41)	474 (49)	633 (52)	409 (51)	26 (41)	1944 (48)
Age (years)						
Male	42.0 (14.6)	45.1 (14.9)	45.5 (15.7)	48.3 (15.5)	46.3 (12.8)	2086
Female	57.1 (8.7)	58.7 (9.4)	60.0 (9.6)	60.6 (9.9)	58.4 (7.6)	1944
BMI (kg/m^2)	24.9 (4.0)	25.3 (4.2)[a]	27.0 (5.1)[b]	26.3 (5.1)[a]	29.0 (4.7)[c]	26.0 (4.7)
Plasma insulin during OGTT (µU/mL)						
0 min	7 (5)	11 (7)[a]	16 (10)[c]	12 (8)[b]	77 (39)[c]	13 (13)
30 min	59 (39)	74 (41)[a]	113 (64)[c]	62 (40)	193 (89)[c]	81 (56)
60 min	70 (48)	95 (51)[b]	161 (76)[c]	86 (55)[a]	224 (96)[c]	109 (72)
120 min	28 (11)	52 (14)[b]	112 (57)[c]	116 (71)[c]	175 (98)[c]	79 (61)
180 min	12 (8)	27 (12)[b]	61 (44)[c]	57 (49)[c]	114 (84)[c]	41 (41)
Plasma glucose during OGTT (mg/dL)						
0 min	86 (10)	87 (10)	87 (11)	85 (10)	84 (13)	86 (10)
30 min	150 (33)	152 (31)	157 (30)[a]	149 (31)	149 (32)	152 (32)
60 min	130 (44)	142 (41)[a]	158 (42)[c]	159 (42)[c]	152 (40)[d]	147 (43)
120 min	86 (21)	99 (18)[c]	108 (18)[c]	115 (17)[c]	104 (24)[c]	102 (22)
180 min	72 (20)	81 (24)[b]	87 (25)[c]	88 (27)[c]	80 (25)	82 (25)
Glucose 120 min – glucose 0 min (mg/dL)	0 (22)	11 (19)[b]	21 (20)[c]	30 (19)[c]	20 (25)[c]	15 (22)
AUCg (mg.hr/dL)	317 (57)	342 (55)[b]	370 (55)[c]	374 (54)[c]	354 (62)[c]	351 (60)
AUCi (µU.hr/mL)	118 (52)	176 (55)[b]	324 (136)[c]	243 (139)[c]	515 (220)[c]	225 (139)

Frequency data are reported as n (%), otherwise mean (SD)

All post-hoc analyses are referenced against Kraft I.

[a] $p<0.001$ and Cohen $d \leq 0.2$, [b] $p < 0.001$ and Cohen d 0.3-0.49, [c] $p < 0.001$ and Cohen $d \geq 0.5$, [d] $p < 0.01$ and Cohen d 0.3-0.49

Although BMI was associated with hyperinsulinaemia in people with normal glucose tolerance, the effect size was modest. What was most notable, was that the majority of people with a hyperinsulinaemic pattern had a BMI < 30kg/m^2, i.e., were not obese. Neither age, nor gender showed an association. The clinical significance of this observation is uncertain, but suggests that the hypothesis that obesity triggers insulin resistance (Qatanani & Lazar, 2007) should be revisited. While not denying that obesity exacerbates insulin resistance and hence hyperinsulinaemia, elevated fasting insulin levels have been shown to precede weight changes in Pima Indian children (Odeleye, De Courten, Pettitt, & Ravussin, 1997). Furthermore, emerging research suggests that insulin changes are associated with, and may precede, weight change (Ludwig & Friedman, 2014; Mehran et al., 2012). This suggests that the relationship between obesity and hyperinsulinaemia may not be unidirectional, but that each condition influences the other in a feedback loop. Therefore, elevated post-prandial insulin levels may be the first symptom of metabolic disease.

The influence of post-prandial hyperinsulinaemia is reinforced by the observation that, with the exception of people with a Kraft IV pattern (fasting insulin ≥ 50μU/mL), there was little clinical difference in the fasting insulin levels between the different Kraft patterns. This was especially noticeable in people with normal glucose tolerance (Table 9). This study shows that fasting insulin should not be relied upon to diagnose hyperinsulinaemia as it has no relationship to post-prandial insulin levels. Future research should consider post-prandial insulin levels, or other metabolic markers that have a clear relationship with post-prandial levels; especially in non-obese people with normal glucose tolerance.

There is a paucity of studies investigating insulin patterns with respect to hyperinsulinaemia in the literature. Hayashi and colleagues investigated the ability of insulin patterns to predict the risk of developing type 2 diabetes over ten years in a cohort of Japanese American men (Hayashi et al., 2013). There are several distinct differences between the two sets of patterns. Hayashi patterns were based on a 75 g, 2-hr oral glucose tolerance test with plasma insulin and glucose sampled at baseline, 30, 60, and 120 minutes. The pattern algorithm is based on the timing of the insulin peaks and troughs. By contrast, Kraft's patterns are based on a 100 g, 3-hr oral glucose tolerance test with similar sampling patterns but the pattern algorithm is based on a combination of the magnitude and the timing of the peaks, plus the rate of decay of the plasma insulin concentration. Despite these differences, there are some notable similarities between the two patterns. Hayashi and colleagues noted that there was a

significantly increased risk of developing type 2 diabetes if the insulin peak was at 2 hours, compared to an insulin peak at 30 minutes. An insulin peak at two-hours equates with a Kraft III pattern. This suggests that those with a Kraft III pattern are at a significantly increased risk of developing type 2 diabetes. Future research should consider whether the addition of peak magnitude enhances the predictive nature of the insulin response patterns.

Although we have no long-term outcomes from this study, it is suggested that an increasing difference between glucose at 120 minutes and fasting glucose is associated with increased risk of cardiac events (Ning et al., 2012). In this study, participants whose 2-hr glucose did not return to baseline had a mean difference between glucose at 120 minutes and fasting levels of approximately 20 mg/dL. Our study shows that people with Kraft III and IV patterns both had a mean difference of ≥ 20 mg/dL.

Our study clearly shows that hyperinsulinaemia is associated with nearly every case of impaired glucose tolerance and type 2 diabetes. Therefore, we contend that everyone with either impaired glucose tolerance or type 2 diabetes should be considered hyperinsulinaemic by default. Although a small proportion of people (2%) with either impaired glucose tolerance or type 2 diabetes also had a Kraft I pattern (normal insulin response) we believe this should be deemed a "pseudo-Kraft I" pattern as the glucose patterns suggest that the pancreas was unable to compensate for the glucose load (Weir & Bonner-Weir, 2004). Furthermore, while it may be argued that people with type 2 diabetes have a 'relative' insulin deficiency (Lebovitz, 1999), once treatment is instigated, these people may become hyperinsulinaemic as indicated by the depiction of the results from a Kraft patterning test, where the person had forgotten to omit their morning insulin dose (Figure 5).

We were surprised that there were small, and potentially non-clinically meaningful, differences in insulin response between people with impaired fasting glucose and those with normal glucose tolerance. This is believed to reflect the difference between insulin-induced hepatic and peripheral insulin resistance which is hypothesised to drive the differences between impaired fasting glucose and impaired glucose tolerance (Abdul-Ghani, Jenkinson, Richardson, Tripathy, & DeFronzo, 2006). The long-term clinical significance of this observation is unknown. However, as people with impaired fasting glucose comprised less than 2% of the complete sample (n = 142), the sample is too small from which to make generalisations and further research is recommended.

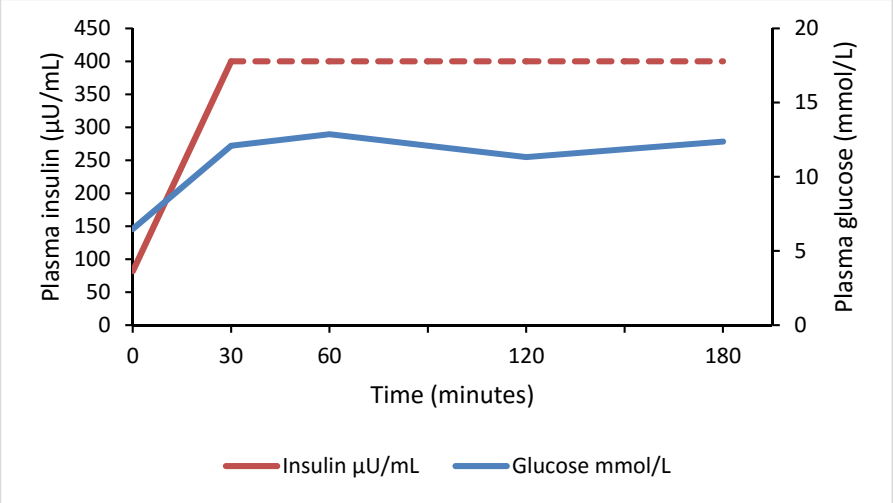

Figure 5: Insulin and glucose response in a patient with type 2 diabetes who received their normal morning exogenous insulin (adapted from Kraft (1994) to aid clarity and preserve patient confidentiality.). The dotted line indicates the maximal range of the test.

There are no current data on the test-retest repeatability of Kraft patterns. There are concerns about the repeatability of oral glucose tolerance tests, especially with respect to gastric emptying, but whether this has a significant effect on the overall insulin response pattern remains unknown (Gordon, Fraser, Bird, & Benson, 2011). Therefore, it is unknown whether the patterns are repeatable with no change in clinical condition. Kraft patterning is based on a 100 g glucose load as this was standard practice in the USA when the data was collected. It is not yet known whether the patterns are repeatable with a 75 g load. These investigations should occur before further research using Kraft patterns are undertaken. However, due to a lack of long term outcome data, the benefits of using all five Kraft patterns remains uncertain, especially when the test is demanding in terms of time and resources. Further research should consider whether a dichotomy of normal/managed insulin response and hyperinsulinaemia can be developed using fewer blood samples, and then applied to long-term outcome data.

This study used data collected up to 40 years ago, therefore, it is uncertain if this represents a modern sample. Although the prevalence of people classified as overweight does not appear to have significantly changed since the 1980s, there has been a sharp increase in adults classified as either obese or extremely obese (Ogden & Carroll, 2010). Additionally, from 1980 to 2011, the prevalence of diabetes has more than tripled (Centers for Disease Control and Prevention, 2013). It is highly plausible that should this study be repeated with a modern population that a much greater prevalence of hyperinsulinaemia would be detected.

Additionally, due to the recruitment methods, there was an unspecified proportion of healthy volunteers to clinically referred participants. This means it is uncertain whether these proportions are representative of the population, with respect to both the prevalence of impaired glucose homeostasis in the total sample, but also with respect to the proportion of people with hyperinsulinaemia in the participants with normal glucose tolerance. Future studies should include concurrent data collection on ethnicity, family medical history, and other metabolic markers to determine if predictive factors for hyperinsulinaemia in the presence of normal glucose tolerance can be more simply obtained.

Current treatment of impaired glucose homeostasis, especially impaired glucose tolerance and type 2 diabetes mellitus focuses on glycaemic control. The impact of this focus on insulin homeostasis of these patients remains uncertain as many people only achieve glycaemic control through the administration of insulin secretagogues or exogenous insulin. Although glycaemic control must be maintained the question remains whether administering high doses of insulin aggravates cardiovascular disease, or increases the risk of developing cancer or dementia (Kelly et al., 2014). Research should explore alternatives to maintaining glycaemic control that minimises insulin requirements; both endogenous and exogenous. For example, carbohydrate-restricted diets provide greater improvements in glycaemic control, weight and other cardiovascular risk factors compared to high-carbohydrate diets, which are the current conventional dietary management of such conditions (Feinman et al., 2015; Kirk et al., 2008). Although the use of insulin sensitisers, such as rosiglitazone, improve peripheral glucose uptake without increasing serum insulin levels (Kahn, Chen, & Cohen, 2000), further research is needed to understand the impact of the increased glucose uptake leading to the increased formation of reactive oxidative species and advanced glycation end-products (Chilelli et al., 2013).

Conclusion

Globally, diseases associated with hyperinsulinaemia are increasing with associated morbidity and socioeconomic burden. In our study cohort, more than 75% of people with hyperinsulinaemia lacked other clinical symptoms, such as impaired glucose tolerance or obesity, therefore suggesting hyperinsulinaemia is a ‘silent disease’. Unlike measures of insulin resistance, insulin response patterns may be useful clinical tools to predict type 2 diabetes. Further prospective research in the benefits of insulin response patterns for disease risk prediction is urgently required to stem the global burden of chronic disease.

Chapter 4: HOMA: Too blunt an instrument?

Preface

Chapter 2 concluded that hyperinsulinaemia should be considered a serious health risk, but more importantly, it should be considered independent to those risks associated with insulin resistance. However, it was also determined that hyperinsulinaemia was poorly defined as the majority of studies investigated insulin resistance using measures such as HOMA or OGIS.

Chapter 3 indicates that a significant proportion of the population may be affected by hyperinsulinaemia. This means that a clinical diagnostic test is urgently required to further advance research and clinical practice in this field. While Kraft I patterns were defined as normal insulin response, assessing the Kraft patterns demands more resources than many of the insulin resistance measures. Given the intertwined nature of insulin resistance and hyperinsulinaemia, it is plausible that insulin resistance measures could assess hyperinsulinaemia. This would allow previous longitudinal research to be reassessed, it would also simplify future research as many of these tests, especially HOMA, are widely used. However, questions remain about the repeatability of fasting insulin, and therefore measures based on fasting insulin, including HOMA. Before HOMA or OGIS can be assessed for diagnosing hyperinsulinaemia, their repeatability must first be assessed.

Traditionally repeatability is assessed as the coefficient of variation (CV) commonly defined as the ratio of the standard deviation to the mean. However, CV is less useful in the clinical field where most clinicians have limited statistical training and want to be able to assess easily whether the latest blood test result indicates a clear change to the patient's clinical condition (improvement or worsening). Repeatability coefficients are an alternative method of assessing repeatability using the calculation:

$$Test\ 1\ \approx Test\ 2\ \pm repeatability\ coefficient$$

Therefore, knowing the repeatability coefficient allows clinicians and researcher a simpler method of assessing clinical change. This technique has never been applied to measures of insulin resistance. This chapter calculates the repeatability coefficient for HOMA2 variants and OGIS to determine whether these measures are sufficiently repeatable for clinical practice.

Abstract

Introduction: The traditional homeostasis model assessment (HOMA) and second generations HOMA2 models are widely used in research to assess insulin resistance despite these measures being potentially insensitive to change due to their high coefficient of variation (CV). Another way of assessing test sensitivity is the repeatability coefficient. To be confident that clinical change has occurred, a subsequent test needs to differ by more than the repeatability coefficient using the equation

$$Test\ 1\ \approx Test\ 2\ \pm repeatability\ coefficient.$$

The repeatability coefficient for measures of insulin resistance are unknown. Therefore, this study will compare the repeatability coefficient of HOMA2 variables (%B, %S, IR) to a dynamic measure of insulin resistance, the oral glucose insulin sensitivity test (OGIS).

Methods: The raw data from a previously used dataset were reanalysed. This included 31 men and women both without (n = 21) and with type 2 diabetes (n = 10) who underwent glycaemic and insulinaemic tests. From this data eight fasting tests and three 50 g oral glucose tolerance tests were used to calculate HOMA2 measures and OGIS. Repeatability was assessed using the methods of Bland and Altman.

Results: Repeatability coefficients for all participants for the HOMA2 %B, %S, and IR measures were 72.91, 189.75, and 0.9, which equates to 89%, 135%, and 89% of their respective grand means. By contrast, OGIS had a repeatability coefficient of 87.13 which equates to 21% of the grand mean.

Conclusion: Due to a lower repeatability coefficient relative to the grand mean, OGIS should be preferred to HOMA2 measures for assessing insulin resistance in small population studies.

Introduction

Homeostasis model assessment (HOMA), and the second generation HOMA2, are methods commonly used to assess changes to insulin resistance / sensitivity resulting from different interventions. Although both HOMA and HOMA2 are practical instruments, they may be too blunt to adequately assess changes to insulin sensitivity in individuals or small populations. To be able to assess change, an instrument needs to have good repeatability. Repeatability is how much variation can be expected among repeat measurements on the same subject under identical conditions. Understanding repeatability enables us to determine if a subsequent test result (e.g. blood test) indicates clinical change or biological variation ("noise"). Repeatability is normally assessed in research by using the coefficient of variation (CV) and expressed by percentage. However, CV may be less useful than the repeatability coefficient. The repeatability coefficient defines the range within which 95% of the differences between two measurements in the same subject by the same measurement method are likely to fall, assuming there is no change in clinical condition between the tests (Bland & Altman, 1999; Hopkins, 2000). Repeatability coefficient can be expressed by the calculation

$$Test\ 1\ \approx Test\ 2\ \pm repeatability\ coefficient.$$ (Bland & Altman, 1999)

Therefore, if Test 2 ± the repeatability coefficient is either larger or smaller than Test 1 , we can be confident that clinical change has occurred. Test variables with a small repeatability coefficient relative to the population mean (Change %) indicate a test that is more sensitive to clinical change. Whereas a test with a large repeatability coefficient relative to the population mean requires a significant degree of clinical change to occur before this will be recognised by the test and is therefore less suitable for clinical use. There are limited repeatability data for most simple insulin resistance measures. The repeatability of HOMA, as assessed by coefficient of variation (CV), ranges from 10 to 50 %. (Lotz et al., 2008; Mather et al., 2001; Widjaja et al., 1999). This may be partially explained by a pulsatile pattern of insulin secretion. The additional challenge with interpreting HOMA (or HOMA2) results is that HOMA is not a single test, but a name to describe a collection of three variables, HOMA %B, HOMA %S and HOMA IR. Although many studies only report the outcome of one variable, it is recommended that at least two variables (HOMA %B and one other) be used to acquire a full understanding of the metabolic state of the participant. Dynamic tests of insulin resistance, such as the oral glucose insulin sensitivity (OGIS) may be less subject to variation. However, there is limited information about repeatability of the

OGIS with just one study reporting a coefficient of variation for duplicate tests as 7.1% (Mari et al., 2001). This study compared the repeatability coefficients for the HOMA2 variables to a dynamic measure of insulin resistance, namely OGIS, in a small group of subjects with or without type 2 diabetes.

Methods

The raw data from Lan-Pidhainy and Wolever (2011) were reanalysed. Ethical permission for this data collection was previously granted by Research Ethics Boards at the University of Toronto and St Michael's Hospital. All participants gave written informed consent.

Subjects and study design

Briefly, 21 healthy participants and 10 participants with type 2 diabetes were recruited. All participants were aged between 18-70 years, had a BMI < 35 kg/m^2; no recent history of hospitalisation; or any history of gastrointestinal, hepatic, or renal disease. All participants with type 2 diabetes used medication: eight used metformin only, one used a combination of metformin and pioglitazone, and one used a combination of metformin and sulphonylurea. These patients took their usual medication on study days after the fasting blood sample but before commencing the test meal.

Lan-Pidhainy and Wolever divided their 21 healthy participants into "control" and "hyperinsulinaemic" sets based on a fasting insulin of 40 pmol/L. By contrast McAuley and colleagues (2001) defined a fasting insulin >73 pmol/L as a "remarkably specific test" for insulin resistance; while Kraft found that a reference range of 0-180 pmol/L could be considered a normal fasting insulin. Due to these discrepancies, we decided to combine the Lan-Pidhainy and Wolever's "control" and "hyperinsulinaemic" sets into a single set termed "No Diabetes". Each participant had fasting blood samples drawn on eight separate mornings. On three of those mornings they then consumed 50 g anhydrous glucose in 250 mL water and on the other five mornings they consumed carbohydrate food (sucrose, instant mashed potato, white bread, polished rice and pearled barley) containing 50 g available carbohydrate. Venous blood samples were then drawn at 15, 30, 45, 60, 90, and 120 minutes for participants without type 2 diabetes and at 30, 60, 90, 120 and 180 minutes for participants with type 2 diabetes. Timing commenced after starting to eat. This study analysed all results from the eight fasting tests and from the three glucose meals.

Blood analysis

Venous blood samples for glucose and insulin were collected in BD vacutainer SST tubes. Serum glucose was measured by the glucose oxidase method (Synchron LX Systems) with inter-assay CV of 1.9%. Insulin was measured using one-step immunoenzymatic assay (Beckman Access Ultrasensitive Insulin Assay) with inter-assay CV of 2.5-4.3%. Insulin has no cross-reactivity with proinsulin.

Calculations and statistical analysis

The glucose and insulin values from each of the three glucose meals were used to calculate OGIS via the available spreadsheet (Mari, n.d.) using only the results from the three glucose test meals. As individual height and weight data were not available for each person, the standards of 1.7m for height and 70kg for weight were used for each person.

The fasting glucose and insulin values from each of the fasting tests were used to calculate each of the HOMA2 measures, (HOMA2 %B, HOMA2 %S and HOMA2 IR,) via the available spreadsheet (Diabetes Trials Unit, 2004).

For each test, within-subject means were plotted against within-subject standard deviations to determine if there was a mean-variance relationship. Ordinary least squares regression was used to assess the strength of such relationships. If the slope coefficient (SC) was significant at the 0.05 significance level, the process was repeated for the mean and standard deviation of the natural log of the variable.

If a significant mean-variance relationship was determined, participants were divided into sub-groups according to test results. The intent was to reduce the mean-variance relationship and therefore bias in the repeatability coefficient at each end of the range while maintaining a clinically meaningful result.

Repeatability was quantified by estimating repeatability coefficients according to the methods of Bland and Altman (1999). Repeatability coefficients were derived from the square root of the residual mean square errors $\sqrt{s_w^2}$ from one-way analyses of variance with subjects as factors fitted to the raw or logged responses for each outcome variable. The 95% repeatability coefficient is $1.96\sqrt{2}s_w$ (Bland & Altman, 1999; Mather et al., 2001).

The following calculations defined the ranges within which two repeat measurements could be expected to fall:

$Test\ 1\ \approx Test\ 2\ \pm repeatability\ coefficient$ for non-log transformed data

or as

$Test\ 1\ \approx Test\ 2\ \times/\div \exp(\quad repeatability\ coefficient)$ for log transformed data

Results

Figure 6 displays the raw data for Control (left) and Diabetes (right) for fasting glucose, HOMA2 %S, HOMA2 %B, HOMA2 IR, and OGIS. Visually, it can be noted that fasting glucose has a narrow spread, especially for the Control participants. By contrast, the spread for all HOMA2 variables is more diverse for both the Control and Diabetes groups. Some participants have a tight cluster of results, while others have a two to four-fold difference in results. The spread for OGIS does not appear to be as tight as that for fasting glucose, but tighter than the HOMA2 variables.

Mean-variance relationships

Mean-variance relationships were positive and significant for all tests with the exception of fasting insulin and HOMA2 IR for the Diabetes group and OGIS for every group (Table 10). Transformation of these variables to their natural logarithm did not remove this relationship for any variable in the "All Participants" group or for the "No Diabetes" group with the exception of the natural log of fasting glucose (Table 10). A significant and positive relationship for fasting glucose for the "No Diabetes" set implied the possibility of subsets within the participant sets and a "Hyperinsulinaemic" set was considered. Our previous research suggests that fasting insulin alone is insufficient to define hyperinsulinaemia (Crofts, Schofield, Zinn, Wheldon, & Kraft, in submission). Examination of the graphed insulin response versus time for all "No Diabetes" participants led to the division of the "No Diabetes" group into "Control" and “Hyperinsulinaemic” (data on file). The latter consisted of three participants who each had the combination of a fasting insulin > 72 pmol/L and a 2-hr insulin > 3x the fasting value. Final sets for analysis were, “All participants", "Diabetes", "No Diabetes" and "Control", with the latter being a sub-set of “No Diabetes”.

Re-examination of the mean-variance relationships identified a non-significant relationship for fasting glucose (p=0.17) and HOMA2 %S (p=0.061) for the Control participants (Table 10). Table 10 demonstrates that, overall, there is little to be gained by using the log-transformed results. With the exception of log fasting glucose for the No Diabetes set, the only measures that demonstrated a non-significant log-transformed mean-variance relationship also had a non-significant mean-variance relationship for the raw data.

Table 10: Regression coefficient and *p*-value for mean-variance relationships

	All participants		Diabetes		No Diabetes		Control	
Variable	Reg Coef	*p*	Reg Coef	*p*	Reg Coef	*p*	Reg Coef	*p*
Fasting glucose (mmol/L)	0.097	<0.001	0.049	0.035	0.165	0.039	0.119	0.17
log fasting glucose	1.474	<0.001	0.54	0.034	2.219	0.069	--	--
Fasting insulin (μU/mL)	0.156	<0.001	-0.23	0.635	0.221	<0.001	0.388	<0.001
log fasting insulin	0.942	<0.001	--	--	0.997	<0.001	1.182	<0.001
HOMA2 %B	0.306	<0.001	0.318	0.001	0.409	<0.001	0.296	<0.001
log HOMA2 %B	0.864	<0.001	1.06	<0.001	1.366	<0.001	1.328	<0.001
HOMA2 %S	0.406	<0.001	0.825	<0.001	0.333	0.007	0.303	0.061
log HOMA2 %S	1.227	<0.001	1.40	0.004	1.220	<0.001	--	--
HOMA2 IR	0.194	<0.001	0.236	0.067	0.180	<0.001	0.363	<0.001
log HOMA2 IR	0.897	<0.001	--	--	0.921	<0.001	1.105	<0.001
OGIS (mL/min/m^2)	0.450	0.099	0.005	0.904	-0.003	0.962	-0.350	0.731

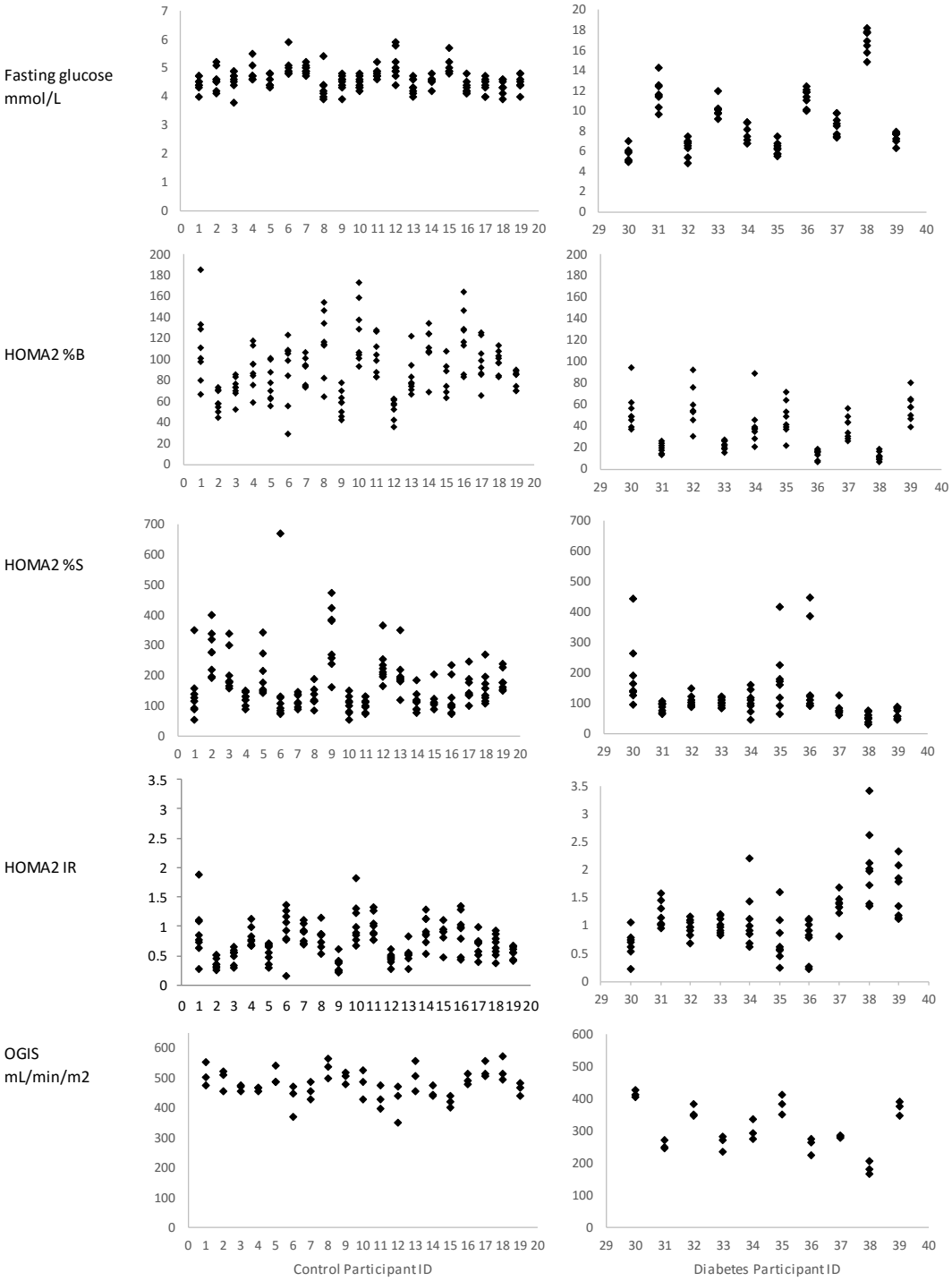

Figure 6: Raw data for control (left) and diabetes (right) for fasting glucose, HOMA2 %B, HOMA2 %S, HOMA2 IR, and OGIS

Repeatability coefficients

Table 11 presents the repeatability coefficients for HOMA2 subtypes, fasting insulin, fasting glucose and OGIS by participant sets. The repeatability coefficient for fasting insulin was 7.91 μU/mL for all participant sets with the exception of the Control subset where it was 5.93 μU/mL. Consistently, the repeatability coefficient was approximately 90% of the participants' fasting insulin Grand mean (supplementary data). The repeatability coefficient for each of the HOMA2 measures ranged between 60% and 170% of their respective Grand means. Within the HOMA2 measures, only HOMA2 IR had a relatively consistent percentage change throughout the participant sets (~ 90%).

OGIS was the only index to have a non-significant mean-variance relationship across all four participant sets (Table 10). The magnitude of the repeatability coefficient was very similar between the Control (96.7 mL/min/m^2) and No Diabetes sets (96.8 mL/min/m^2), but markedly different to the Diabetes set (60.7 mL/min/m^2) (Table 11, Supplementary information). However, this represents a relatively consistent percentage change of approximately 20%.

Discussion

Our study examined the repeatability coefficient for the HOMA2 variables and OGIS in people both with, and without type 2 diabetes. The results show that although the repeatability coefficients vary by participant subset, measures based on fasting insulin, including all HOMA variables, require a large change relative to the population mean in order to detect clinical change. This means that a subsequent test of either fasting insulin, HOMA2 %B, or HOMA2 IR needs to differ from a former test by approximately 90%, with the exception of HOMA2 %B for people with normal glucose and insulin tolerances (65%). HOMA2 %S needs to differ by approximately 120%. Conversely, only a 15-20% difference is needed for OGIS, suggesting that the change is due to clinical condition rather than biological variation.

These two key findings from this study suggest that for testing individuals or small populations OGIS should be the preferred insulin resistance test compared to either fasting insulin or any variant of HOMA2.

Table 11: Repeatability coefficients for simple measures of insulin resistance (all data).

	All participants (n = 31)				Diabetes (n = 10)				No Diabetes (n = 21)				Control (n = 18)			
Variable	s_w	±Rep Coef	$\hat{\mu}$	Change %	s_w	±Rep Coef	$\hat{\mu}$	Change %	s_w	±Rep Coef	$\hat{\mu}$	Change %	s_w	±Rep Coef	$\hat{\mu}$	Change %
Fasting glucose (mmol/L)	0.59	1.62	6.08	26.64	0.93	2.59	9.20	28.15	0.32	0.88	4.65	18.92	0.30*	0.84	4.06	20.69
Fasting insulin (µU/mL)	2.85	7.91	8.61	91.87	2.86*	7.90	8.59	91.97	2.85	7.90	8.62	91.65	2.14	5.93	6.52	36.09
HOMA2 %B	26.31	72.91	82.31	88.57	13.08	36.26	36.41	99.45	30.53	84.61	103.18	82.00	20.67	57.31	90.62	63.24
HOMA2 %S	68.46	189.75	140.05	135.49	70.02	194.09	111.19	174.56	67.72	187.72	153.17	122.56	72.68*	201.45	169.33	118.97
HOMA2 IR	0.32	0.90	1.01	89.11	0.39*	1.07	1.15	93.04	0.29	0.81	0.95	85.26	0.23	0.64	0.72	88.89
OGIS (mL/min/m^2)	31.43*	87.13	413.10	21.1	21.88*	60.67	303.69	19.98	34.91*	96.77	462.84	20.91	34.90*	96.74	475.79	20.33

s_w = residual mean square error; Rep Coef = repeatability coefficient; $\hat{\mu}$ = Grand mean

* Denotes a non-significant mean-variance relationship from Table 10

OGIS was conducted with 3 repeated tests. All other variables had 8 repeated tests

It was our intention that the relevance of this study was geared more towards practice rather than research. HOMA variants are used widely in exercise science research to assess effectiveness of interventions aimed at improving insulin sensitivity. However, measurements of insulin resistance are discouraged in medical practice due to a lack of effectiveness in disease risk calculations (Samaras et al., 2006). This study aimed, in part, to try and understand this discrepancy. Using the repeatability coefficient, rather than the more commonly used coefficient of variation, was also a deliberate choice aimed at practice. Using the repeatability coefficient allows us to easily interpret whether the differences between two measures are biological variation ('noise'), or clinical change. For the same reasons, we chose to include the analytical variation as part of the within-subject variation.

There is a paucity of data on the test-retest repeatability of measures of insulin resistance, especially those reporting on repeatability coefficient. HOMA-IR has been reported as needing to change by +90% or -47% in patients with type 2 diabetes to ensure that the second sample is clinically significant when compared to a previous sample (Jayagopal, Kilpatrick, Jennings, Hepburn, & Atkin, 2002). As this study used the original HOMA model, we cannot directly compare results, however, both studies show that large changes are needed in HOMA-IR in people with diabetes to ensure that there is clinical change. The degree of change that is required suggests that HOMA-IR is an impractical clinical measure in people with diabetes.

Using repeatability coefficient rather than coefficient of variation meant that it was harder to compare our results to the existing literature. CV can be derived from the repeatability coefficient using the following formula:

$$CV \approx \frac{s_w}{\hat{\mu}} \approx \frac{Rep\ Coef}{2.77 \times \hat{\mu}}$$

Using this conversion, our results align with current CV reports. Gordon and colleagues reported the CV for OGIS to be 7.8% (range 4.2-14.2 %) for 8 people with four repeated tests (2011). This compares to our CV of approximately 7%. Widjaja and colleagues reported a within-subject CV for fasting insulin of 26% (1999) for daily measures taken over 12 days. This is comparable to our findings of 32%. Higher CV results for HOMA were also noted by Mather and colleagues, who reported a CV of 58% for HOMA-IR in subjects with a BMI >

27 kg/m^2 compared to 24% in subjects with a BMI of < 27 kg/m^2 (2001). This compares to our CV of 32% for HOMA2 IR.

Along with the overall paucity of data, another challenge with comparing repeatability studies involves the very different methodologies available. Insulin concentrations will vary between studies depending on whether the study used plasma or serum and which analytical method was involved. (Henderson, 1970; Manley, Stratton, Clark, & Luzio, 2007). Participant factors such as age, sex, body fat distribution, and health status may also affect insulin sensitivity (Karakelides, Irving, Short, O'Brien, & Nair, 2010). It remains unknown whether repeatability is consistent amongst all these different groups. Repeatability studies may also focus on biological variation by excluding analytical variation from the overall variation (Widjaja et al., 1999). While excluding analytical variation may be useful in the research paradigm, it is impractical in practice. The number of repeated tests also varied with some studies using duplicated measures, while others used three or more measures. These factors may explain why some studies show good repeatability for HOMA variables, while others show a much wider variation. The differences in these factors may also impede direct comparisons or generalisations amongst studies.

Furthermore, the repeatability coefficient changes depending on the subset of the population. A notable finding of our study was the maintenance of a positive and significant mean-variance relationship for almost all of the study variables, including fasting glucose; OGIS was the only variable that consistently lacked a positive and significant mean-variance relationship. These positive mean-variance relationships mean that the repeatability coefficient may be over- or under-estimated at the extremes of the ranges of observed test results. Given that measures based on fasting insulin required 60-175% difference in results to ensure clinical change, the influence of the bias may not matter. What was clear is that OGIS did not have a positive and significant mean-variance relationship for any sub-grouping tested, and although the repeatability coefficient altered depending on the sub-group, it remained a consistent 20% of the population grand mean.

Although we calculated the absolute figures for the repeatability coefficient, these were converted to percentages to determine if there were consistencies throughout the sub-groupings. (Table 11). It was believed that should a consistency be found, then percentages may be more practical as a) fewer figures would need to be remembered, and b) the patient would not have to be sub-classified accurately.

The results from our study suggest that HOMA2 measures are not sufficiently accurate to detect small changes in clinical condition in the individual. The implications of HOMA2 variability for larger scale research projects are not yet known. Considering the use of insulin resistance testing, in exercise science research to either classify participants or assess the effects of an intervention, these results suggest that using HOMA to classify participants may not be effective as participants are likely to have different results on different testing occasions. If HOMA variables are to be used as a primary outcome, then power calculations should be conducted to ensure that the study has a sufficient sample size in order to accurately detect change. Many studies do not use HOMA as a primary outcome, and this would be reflected in sample size. In a placebo-controlled intervention study, in order to detect a 15% change in HOMA2 IR in people with normal glucose tolerance, a target sample size of 55 people in each arm is needed to provide 80% power at the 0.05 level of significance using a two sample t-test. This assumes our detected standard deviation of 0.28 applies to both arms. In people with type 2 diabetes, the increased standard deviation of 0.38 then requires a target population of 100 people in each arm. Many studies do not have these participant numbers, therefore, we cannot be confident that the documented changes in HOMA variables resulting from different interventions are legitimate outcomes.

We accept that measures based on fasting insulin are much cheaper and less demanding than those based on the results derived from an oral glucose tolerance test. This may, in part, explain the popularity of HOMA. We further recognise that only recommending tests based on an oral glucose tolerance test would likely result in fewer assessments of insulin resistance. But should we settle for this and compromise accuracy for convenience? Given there is still little practical value in measuring insulin resistance it may be that the resources are better used elsewhere.

The large number of repeated tests of fasting measures (n = 8) was a particular strength for our study. We were also able to assess these measures in people both with type 2 diabetes (n = 10) and with normal glucose tolerance (n = 21). There were a number of limitations to our study. OGIS has only been validated against the hyperinsulinaemic-euglycaemic clamp test for the glucose 75 g, 3-hr test (Mari et al., 2001). This study used 50 g glucose and while the participants with diabetes had a 3-hr test, those without diabetes only had a 2-hr test. The original participant height and weight data was no longer available. Therefore we applied a standard height and weight for each participant. Although this would not have affected the

within-subject variability, it would have reduced the between-subject variability. People with diabetes took their regular medication as part of the study. While this reflects their normal post-prandial response, the medication plausibly decreased the within-subject variation.

Conclusion

Although HOMA measures are a convenient test to use to assess insulin resistance, their high variability precludes accuracy in diagnosing change at both the individual and research level. A subsequent test needs to change by approximately 90% to be confident that clinical change has occurred. Dynamic measures such as OGIS are less popular due to higher resource requirements, but they have a significantly higher degree of repeatability. The question still remains as to whether insulin resistance should be measured in research or practice, but dynamic measures should be preferred to fasting measures.

Chapter 5: Assessing the repeatability characteristics of insulin response patterns and measures of insulin resistance.

Preface

So far, this thesis has highlighted that hyperinsulinaemia is an independent health risk, but clinical diagnosis is currently limited (Chapter 2). Kraft patterns, as shown in Chapter 3, can indicate normal, hyper-, and hypoinsulinaemic insulin response patterns when derived from multiple blood samples during an oral glucose tolerance test, (also known as dynamic testing). This data was derived from cross sectional data, so there are no longitudinal outcomes. As fasting measures of insulin resistance, such as HOMA, are currently preferred, there is limited data from which to derive these data. It was hypothesised that insulin resistance measures may be able to predict insulin response patterns, but there were concerns about the test-retest repeatability of these measures. Chapter 4 demonstrates that fasting measures of insulin resistance, as illustrated by using the HOMA variable, are insufficiently sensitive for clinical use. The dynamic measures, OGIS, was sufficiently sensitive.

Currently there is no test-retest repeatability data for Kraft or Hayashi patterns and only limited data for OGIS. Since the data so far indicates that hyperinsulinaemia needs dynamic testing, this gap in the literature needed to be rectified.

Abstract

Introduction

Hyperinsulinaemia is emerging as an independent risk factor for metabolic disease, but diagnostic measures are limited. It is plausible that insulin resistance measures such as HOMA2 variants, OGIS and the McAuley Index may model hyperinsulinaemia, but data is lacking on the repeatability of these measures. Kraft and Hayashi insulin response patterns may add value in diagnosing hyperinsulinaemia, but also lack suitable repeatability data.

Methods

Oral glucose (100 g) tolerance tests were conducted weekly on eight people. Six people completed four tests while the remaining two completed at least two tests. For each test insulin resistance and insulin response patterns were assessed and compared between weeks. Insulin resistance measures included fasting tests (HOMA2 variants and the McAuley Index) and the dynamic test, OGIS. The insulin response patterns were assessed according to the methods of both Kraft and Hayashi. Repeatability characteristics of ordinal variables were assessed according to methods of Bland and Altman while Fleiss' kappa was applied to categorical variables.

Results

Fasting measures of insulin resistance recorded poor repeatability (HOMA2 variants) or poor sensitivity (McAuley Index) compared to the dynamic measure OGIS. Kraft insulin response patterns were more repeatable compared to Hayashi patterns, based on a combination of Fleiss' kappa (0.290 vs 0.186,) *p*-value (0.15 vs 0.798) and 95% confidence intervals.

Conclusions

Both hyperinsulinaemia and insulin resistance should be assessed dynamically following an oral glucose tolerance test. Kraft patterns should be the preferred dynamic insulin patterning methodology for hyperinsulinaemia due to their higher repeatability.

Introduction

Insulin resistance is recognised as being a significant risk factor for type 2 diabetes and other metabolic diseases. Yet insulin resistance measures do not add value to disease risk calculations (Samaras et al., 2006; Schmiegelow et al., 2015). People with insulin resistance generally have chronic hyperinsulinaemia to compensate for the poor glucose uptake rates. This compensatory hyperinsulinaemia, as recognised as an independent risk factor for metabolic disease (Chapter 2), may be one of the earliest indicators of incipient disease. Yet there is no consistency in its quantification. Since hyperinsulinaemia coexists with insulin resistance, it is plausible that insulin resistance measures may also predict hyperinsulinaemia.

The gold-standard method for assessing insulin resistance is the hyperinsulinaemic-euglycaemic clamp. However, this method is not practical in the clinical or large-scale research settings so alternative methods are used that model the clamp. These alternative methods include fasting tests such as homeostasis model assessment (HOMA) and the McAuley index. "Dynamic" methods are based on results derived from a combination of fasting and post-prandial testing during an oral glucose tolerance test (OGTT) and include oral glucose insulin sensitivity (OGIS).

Despite being widely used, there is limited information regarding population normative values of insulin resistance, with many studies defining insulin resistance as a quantile of the population under investigation. One explanation for poor predictive value compared to other measures is that fasting insulin resistance measures have a high variability. For example, as shown in Chapter 4, HOMA and HOMA2 variants, have both a high CV (25-50%) and a large repeatability coefficient proportional to the population mean (89-135%). This is likely related to the known variability of fasting insulin (Wallace et al., 2004). Repeatability data for the McAuley index is lacking. The dynamic insulin resistance measure, OGIS, has a lower degree of variability as indicated by CV (8%) and repeatability coefficient proportional to the population mean (22%).

Evidence is limited for assessing hyperinsulinaemia, especially given the high variability of fasting insulin. Emerging research proposes using insulin response patterning via a multiple-sampled OGTT. Kraft (1975) described five distinct insulin patterns formed during a three-to-five hour OGTT on the basis of magnitude and timing of the peak plasma insulin level rate of decay. A normal insulin response was

considered to be a fasting insulin ≤ 30 μU/mL, with a moderate peak 30-60 minutes after the glucose load and a rapid rate of decay. Independently in their sample of 400 Japanese American men, Hayashi and colleagues determined that an insulin peak at 120 minutes during a 2-hr OGTT, significantly increased the risk of developing type 2 diabetes over the following ten years (2013).

Assessing insulin response patterns is expensive as they require four to five blood samples over a two to three-hour time period. It is plausible that insulin resistance methods may be able to predict hyperinsulinaemia given the two conditions are intertwined. However, to have clinical utility, tests need to have a low variability. There are concerns about the variability of insulin resistance measures, and the repeatability of insulin response patterns is unknown.

The aims of this study are two-fold. Firstly, to assess the test-retest repeatability of fasting and dynamic insulin resistance measures, and that of dynamic insulin response patterns. It also aims to determine whether insulin resistance measures can predict hyperinsulinaemia.

Methods

Subjects and study design

We recruited ten healthy participants (six male, four female), aged 20-55 years. Each participant had an HbA_{1c} < 40 mmol/mol, and had no acute or chronic injury or illness requiring medical attention in the previous three months. Participants were required to adhere to the standard oral glucose tolerance testing procedures including no vigorous exercise on the morning of the test. As per previous Kraft patterning protocols, all participants were asked to consume at least 150 g carbohydrate per day for at least 14 days prior to the first test and to maintain this level of carbohydrate consumption throughout the testing period (Kraft, 1975). Participants were also asked to maintain their normal physical activity patterns throughout the two-week lead-in and four-week study protocol period. No formal assessment was made of diet or physical activity. This was a deliberate decision as it was believed that this would more closely reflect clinical practice. The clinical criteria chosen and the short study time was designed so that it would be unlikely that an underling clinical condition could influence insulin responses and confound the results.

On the first test occasion, height, weight and waist girth (smallest girth between the lower rib and iliac crest) was measured. After an overnight fast, each subject had a cannula inserted into their antecubital fossa and provided fasting venous blood samples before consuming 100 g glucose (400 mL Carbotest™ solution). The glucose drink was consumed within 10 minutes of test commencement (0 minutes). Further venous samples were drawn at 30, 60, 120, and 180 minutes. Vein patency was maintained by flushing with saline before and after each collection, with the first 2mL of blood collected being discarded. This protocol was repeated weekly for a total of four tests with the exception of HbA_{1c} which was assessed on the first week only.

Analysis

Sample analysis

Venous samples were collected from the antecubital fossa in PST and EDTA vaccutainers (Becton, Dickinson and Company, Franklin Lakes, NJ). The whole blood EDTA samples were analysed for HbA_{1c} (Roche Cobas C111, tubidimetric inhibition immunoassay with interbatch CV of 1.32-2.36%). Plasma was extracted from the PST tubes after centrifugation (1500 rcf at 4°C for 10 minutes), then frozen at -20°C within 2 hours of collection. Prior to analysis, plasma samples were allowed to warm to room temperature and centrifuged (10 000 rcf at 20 °C for 30 seconds) to remove any protein precipitants. Samples were batch-analysed by participant to reduce intermediate precision. All plasma samples were quantitated on the Roche Diagnostics cobas Modular Analytics E170. Insulin was quantitated on the E module via electrochemiluminescence (intermediate precision 2.5-4.9%). All other analytes were quantitated on the P module: Glucose was quantitated via the hexokinase enzymatic method (intermediate precision 1.7-1.9%); triglycerides via an enzymatic colorimetric method (intermediate precision 1.8-2.4%); and CRP via particle enhanced immune-turbidimetric assay (intermediate precision 0.5-2.0%). Where possible automated haemolysis index measured quantified haemolysed samples. Samples were excluded from further analysis if significant haemolysis was present.

Calculations and statistical analysis

Statistical analysis and calculations were performed with either SPSS 22.0 (Armonk, NY) or Microsoft Excel 2013 (Redmond, WA). The following measures were calculated for each weekly test: HOMA2 %B, HOMA2 %S, HOMA2 IR; OGIS; McAuley Index; Hayashi pattern; Kraft pattern; and WHO glucose tolerance testing.

HOMA2 and OGIS were calculated using their respective downloadable calculators (Diabetes Trials Unit, 2015; Mari, n.d.)

Two group comparisons were made with two-tailed independent t-tests. Missing data was imputed as according to the most likely clinical scenario for pattern reconstruction and Fleiss's kappa (κ) calculations only.

McAuley Index was calculated using the following formula:

$Mffm/I = \exp[2.63 - 0.28\ln(fasting\ insulin) - 0.31\ln(fasting\ triglycerides)]$ (McAuley et al., 2001).

Kraft and Hayashi patterns were derived following their respective protocols (Crofts, Schofield, et al., in submission; Hayashi et al., 2013); Kraft I pattern is considered to be normal insulin tolerance (Kraft, 1975). Glucose tolerance testing followed WHO protocols (World Health Organization, 2006). Insulin and glucose response curves collected over repeat visits were summarised by plotting point-wise arithmetic mean concentrations for each participant.

Test-retest repeatability measures

Fleiss' κ was calculated as a means of assessing pattern repeatability for both Kraft and Hayashi patterns (1971). As there is no standard interpretation of κ, significant agreement for the pattern was considered to be a combination of Landis and Koch's recommendations (1977), significance of κ, and whether the 95% confidence intervals crossed zero.

For insulin resistance measures, repeatability was quantified by estimating repeatability coefficients according to the methods of Bland and Altman (1999). As this method assumes a non-significant means-variance relationship, within-subject means were plotted against within-subject standard deviations to determine if there was a mean-variance relationship. Ordinary least squares regression was used to assess the strength of such relationships. If the slope coefficient (SC) was significant at the 0.05 significance level, the process was repeated for the mean and standard deviation of the natural log of the variable.

If a significant mean-variance relationship was determined, participants were divided into sub-groups according to test results. The intent of these sub-groups was to reduce

the mean-variance relationship and therefore bias in the repeatability coefficient at each end of the range while maintaining a clinically meaningful result.

Repeatability coefficients were derived by taking the square root of the residual mean square errors (s_w) from one-way analysis of variance with subjects as factors fitted to the raw or logged responses for each outcome variable. The 95% repeatability coefficient is $1.96\sqrt{2}s_w$ (Bland & Altman, 1999; Mather et al., 2001). Ranges within which two repeat measurements could be expected to fall were defined as Test 1 ≈ Test 2 ± repeatability coefficient for non-log transformed data or as Test 1 ≈ Test 2 ×/÷ exp(ln repeatability coefficient) for log transformed data. CV was derived from the repeatability coefficient using the formula $CV \approx \frac{s_w}{\hat{\mu}}$.

Results

Ten participants consented to the study, but only eight participants completed at least two tests. The baseline characteristics of these eight participants are displayed in Table 12. Results were included for all participants who completed at least two tests. Six participants completed all four tests, one participant (K10) could not attend on one occasion, and one participant (K6) was unable to adhere to fasting requirements on two occasions.

Table 12: Participant characteristics

Code	Sex	Age (years)	Height (m)	Weight (kg)	BMI (kg/m2)	Waist (m)	W:H	HbA1c (mmol/mol)
K1	M	47	1.744	81.8	26.9	0.872	0.50	32.4
K2	M	53	1.737	81.8	27.1	0.956	0.55	35.4
K3	M	44	1.726	74.0	24.8	0.810	0.47	35.8
K4	F	29	1.721	71.0	24.0	0.792	0.46	37.5
K5	F	39	1.515	60.0	26.1	0.755	0.50	36.5
K6	M	30	1.634	65.9	24.7	0.832	0.51	34.2
K9	M	31	1.852	91.6	26.7	0.823	0.44	32.8
K10	M	27	1.774	76.7	24.4	0.804	0.45	35.8

Figure 7 displays the mean insulin and glucose response curves for each participant. A higher peak and/or delayed rate of decay can be observed for participants K2, K5 and K6.

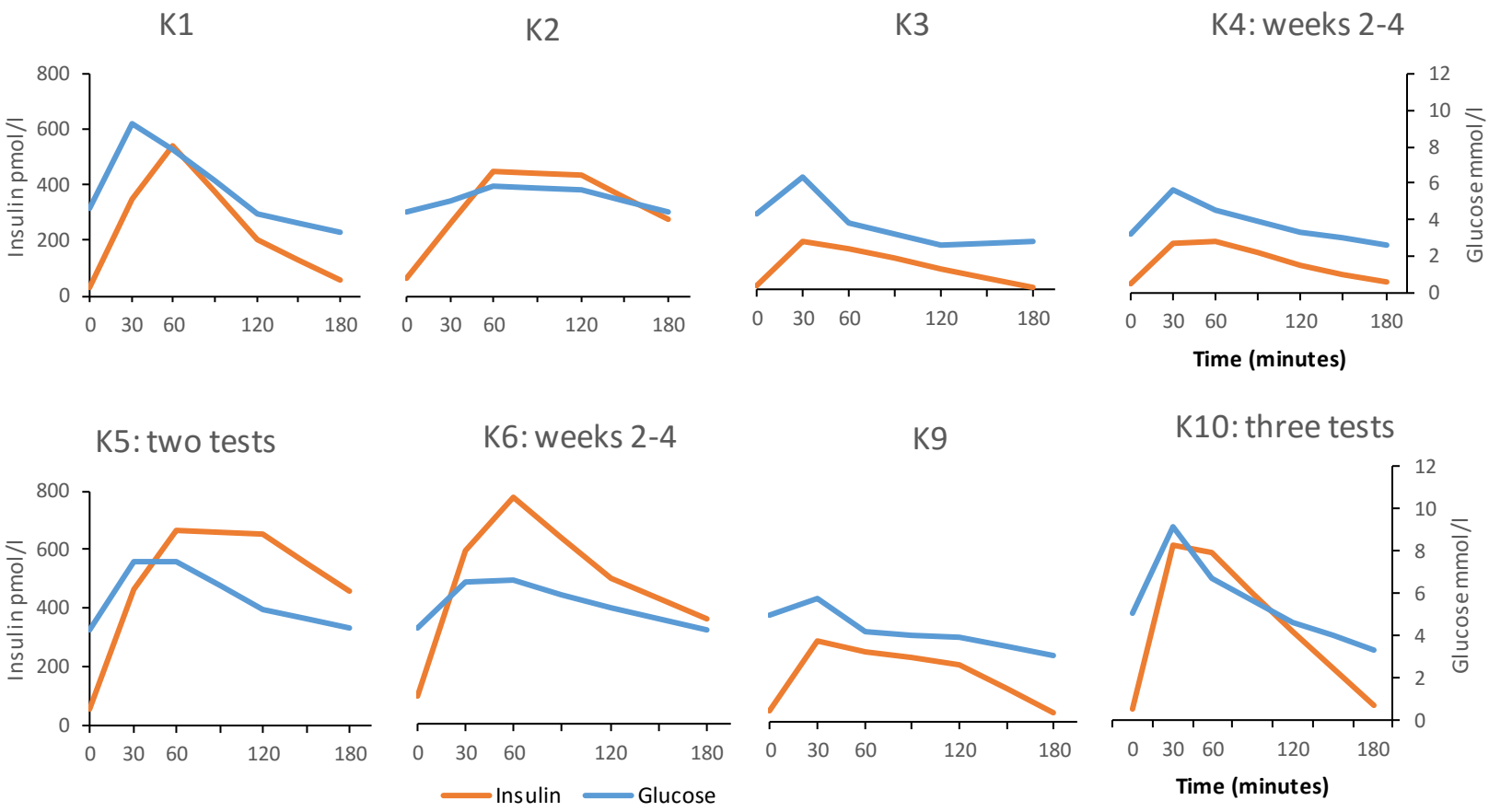

Figure 7: Point-wise arithmetic mean insulin (pmol/L) and glucose (mmol/L) concentrations for each participant. Participants K4 and K6 both had week 1 results excluded: K4 due to an elevated CRP and K6 due to extensive haemolysis of the 60-minute sample.

Repeatability coefficient for insulin resistance measures

Mean variance relationships could only be detected for fasting glucose, fasting insulin and glucose at 180 minutes. After the removal of participant K4 from the dataset, a mean variance relationship could no longer be detected for either fasting glucose or glucose at 180 minutes. Log-transformation of fasting insulin did not remove the mean variance relationship. No mean variance relationship could be detected for fasting insulin for the subset of hyperinsulinaemic participants (K2, K5, and K6).

Table 13 displays the repeatability coefficients for all time points for insulin and glucose; the McAuley index; all HOMA measures; and OGIS. There was no practical difference in the repeatability coefficient for glucose at 180 minutes when participant K4 was excluded (Rep Coef = 2.24, p <0.001). Among the fasting models of insulin resistance, the McAuley index had the lowest repeatability coefficient compared to the grand mean of the sample (17.4%).

Table 13: Repeatability coefficients for all participants

	s_w	±Rep Coef	$\hat{\mu}$	Change %	CV %
Glucose 0 min mmol/l	0.27	0.74	4.81	15.4	5.5
Glucose 0 min (without K4) mmol/l	0.20	0.56	4.86	11.5	4.2
Glucose 30 min mmol/l	1.02	2.81	7.43	37.8	13.7
Glucose 60 min mmol/l	1.83	5.08	6.00	84.7	30.5
Glucose 120 min mmol/l	1.33	3.68	4.94	74.5	26.9
Glucose 180 min mmol/l	0.80	2.23	3.94	56.6	20.4
Insulin 0 min[a] pmol/l	11	31	44.42	68.9	24.8
Insulin 30 min pmol/l	101	279	348.94	80.0	28.9
Insulin 60 min[b] pmol/l	178	494	415.16	119.0	42.9
Insulin 120 min pmol/l	102	282	294.38	95.8	34.6
Insulin 180 min pmol/l	71	197	152.83	129.0	46.5
McAuley Index Mffm/I	0.35	0.98	5.62	17.4	6.3
HOMA2 %B	14.2	39.5	95.66	41.3	14.8
HOMA2 %S	26.1	72.4	129.56	55.9	20.1
HOMA2 IR	0.24	0.67	0.89	75.4	27.1
OGIS mL/min/m^2	27.5	76.1	514.19	14.8	5.3

s_w = residual mean square error; Rep Coef = repeatability coefficient; $\hat{\mu}$ = grand mean
[a] Significant mean variance relationship
[b] excluding K6, week 1 due to haemolysis

Repeatability of insulin response patterns

Table 14 presents the distribution of each test per participant for both Kraft and Hayashi insulin response patterns. The most common Kraft pattern was pattern I, recorded by five of the eight participants, while the most common Hayashi pattern was pattern 3 (eight of eight participants). No participant recorded a Kraft IV or V pattern, or a Hayashi pattern 5. Three participants (K5, K6, and K10) were initially excluded from κ calculations as they did not have four eligible tests for both pattern responses. However, small participant numbers meant that missing data decreased the power of the study.

Table 14: Raw data of Kraft and Hayashi pattern frequencies on 8 participants over four visits per participant.

	Kraft pattern						Hayashi pattern				
Participant	I	IIA	IIB	III	IV	V	1	2	3	4	5
K1	4								4		
K2			2	2					2	2	
K3	4						3		1		
K4	3			1			2		1	1	
K5			1	1					1	1	
K6*		1	3						3		
K9	4							2	2		
K10	2	1					1		2		

K6: The week 1, 60-minute result was extensively affected by haemolysis. While this did not affect Kraft patterning, the Hayashi pattern could not be determined.

Therefore, we replicated the repeatability calculations after imputing the clinically most likely, or most frequent clinical outcome for participants with missing data (K5, K6, and K10) as shown in Table 15.

Table 15: Kraft and Hayashi pattern frequencies on 8 participants over four visits per person using imputed data to account for missing results.

	Kraft pattern						Hayashi pattern				
Participant	I	IIA	IIB	III	IV	V	1	2	3	4	5
K1	4								4		
K2			2	2					2	2	
K3	4						3		1		
K4	3			1			2		1	1	
K5			2	2					2	2	
K6		1	3						4		
K9	4							2	2		
K10	3	1					2		2		

	Explanation		Explanation
K5	One test each added to pattern IIB and III as there was previously a 50:50 split.	K5	One test each added to patterns 3 and 4 as there was previously a 50:50 split.
		K6	The week 1, 60-minute result was extensively affected by haemolysis. Extrapolation of the raw data suggested a 60 minute peak was the most likely scenario, therefore pattern 3.
K10	One test added to pattern I as this was a) the most common pattern, and b) the pattern IIA was associated with a sub-acute change to normal clinical state.	K10	Unable to extrapolate from raw data whether a pattern 1 or 3 was most likely. Both scenarios run, with negligible difference to κ.

The inclusion of the imputed data did not cause a substantial change to the overall results as shown in Table 16. Estimated kappa for the Kraft patterns was higher than for Hayashi patterns (0.290 vs. 0.186) but only the kappa for the Kraft patterns was significantly different from zero (95% CIs: (0.515, 0.798) and (-1.238, 1.610) for Kraft and Hayashi respectively).

Table 16: Fleiss' kappa calculations for raw and imputed data

	Kraft patterns		Hayashi patterns	
	Raw data	Imputed data	Raw data	Imputed data
p-value	0.015	< 0.001	0.798	0.347
κ	0.290	0.417	0.186	0.451
95% CI				
upper	0.622	0.532	1.610	1.392
lower	0.267	0.230	-1.238	-0.489

Characteristics of insulin resistance measures compared to insulin response patterns

Table 17 displays the participants' insulin resistance measures when dichotomised into normal (Kraft I) and hyperinsulinaemic (Kraft IIA, IIB, III) insulin response patterns. Statistically significant differences can be noted for HOMA2 measures and for OGIS, but not for the McAuley index.

Table 17: Insulin resistance measures compared to insulin response patterns.

	Kraft I (n= 5)		Kraft IIA, IIB, III (n = 3)		
	Mean	SD	Mean	SD	*p*-value
McAuley index Mffm/I	4.99	0.82	4.51	0.46	0.095
HOMA2 %B	73.87	19.70	121.11	16.09	<0.001
HOMA2 %S	183.93	52.96	82.43	20.34	<0.001
HOMA2 IR	0.58	0.21	1.28	0.35	<0.001
OGIS mL/min/m^2	547.49	52.86	450.92	28.18	<0.001

Discussion

Numerous tests are available for assessing insulin resistance and may be based on either fasting measures or dynamically modelled from oral glucose tolerance tests. Tests based on fasting insulin such as HOMA and HOMA2 variants are popular as they require fewer resources compared to those based on dynamic testing (e.g. OGIS). There is also a need to assess hyperinsulinaemia as it is now recognised as an independent disease risk predictor. However, a lack of repeatability testing for both insulin resistance and hyperinsulinaemia measures precludes their clinical use. This study assessed the repeatability characteristics of the fasting measures: HOMA2 variants and McAuley Index; and the dynamic measure OGIS by comparing each repeatability coefficient to the cohort grand mean. We also assessed the repeatability of the two insulin response patterns, Kraft and Hayashi patterns using Fleiss' kappa.

Repeatability of insulin resistance measures

Of the insulin resistance measures (HOMA2, McAuley and OGIS), only the McAuley Index and OGIS demonstrated a low repeatability coefficient relative to the grand mean of the sample population with % change of 17.4 and 14.8% respectively. By contrast HOMA2 variants had a higher % change (HOMA2 %B = 41.3%, HOMA2 %S = 55.9%, and HOMA2 IR = 75.4%). These HOMA2 findings are comparable to our previous research in a population of people with normal glucose tolerance (Crofts, Wheldon, et al., in submission).

Most studies assess repeatability using CV. While it may not be possible to directly compare the repeatability of the original HOMA model with the HOMA2 model, our findings (HOMA2 %B = 14.8%, HOMA2 %S = 20.1%, and HOMA2 IR = 27.1%) align with CVs reported from the original model including that of Mather and colleagues who reported HOMA IR having a CV of 24% (Mather et al., 2001). CV data for the McAuley Index is limited with one study reporting a CV of 15.1% (Sarafidis et al., 2007). This is higher than our finding of 6.3%.

Repeatability of insulin response patterns

The OGTT has a few, mixed, reports for repeatability yet it is a very common clinical test (Gordon et al., 2011). Few studies have investigated the repeatability or reproducibility of insulin response curves; of those that have, no significant differences in $AUC_{insulin}$ have been noted (Gordon et al., 2011; Utzschneider et al., 2007). There are no published studies that have assessed the repeatability of insulin response patterns, namely the Kraft and Hayashi patterns. Our study demonstrated that the Kraft pattern methodology had a higher reproducibility and were more likely to provide a consistent pattern following multiple OGTT when compared to the Hayashi patterning methods. Kraft patterns account for both the magnitude of the insulin response and rate of decay as well as the timing of the insulin peaks. By contrast Hayashi patterns only consider the timing of the insulin peaks, and thus accord less information. This suggests that the magnitude of the insulin response as well as the rate of decay should also be considered when assessing insulin patterns.

Consistency amongst insulin response pattern was more common for participants who were predominantly Kraft I pattern (n=5). Of the two participants who deviated from Kraft I pattern, the first (K4) had a moderately elevated CRP the same week of their deviation (week 1); they reverted to a consistent Kraft I pattern with the resolution of

the elevated CRP. The second was consistently Kraft I pattern until the final week when they demonstrated a Kraft IIA pattern. This participant admitted to feeling more stressed during that week's test compared to the other weeks so possibly had an elevated cortisol level compared to previous weeks. It is known that cortisol can induce a hyperinsulinaemic response (Björntorp & Rosmond, 1999). Although we did not measure cortisol it is clinically plausible that it was elevated in both of these situations. This suggests that insulin response patterning should only be conducted during times of stable clinical condition.

For those participants who never exhibited a Kraft I pattern (n=3), consistency amongst patterns was lower. Two participants exhibited a 50:50 split between patterns IIB and III, while the third was predominately pattern IIB, with one occasion of pattern IIA. Unlike the participants who deviated from a predominant Kraft I pattern, there was no clear plausible clinical indication for these variations. This may indicate that these hyperinsulinaemic states are more transitory than a normal insulin response (Kraft I). Although a larger study will be needed to confirm these results, it appears that the Kraft patterns are sufficiently reproducible to confirm Kraft I pattern or normal insulin status, or "not" which would generally be a hyperinsulinaemic status (Kraft IIA-IV patterns).

Variation was higher within the Hayashi patterns. Every participant exhibited a Hayashi 3 pattern at least once. Most (75%) also exhibited either a Hayashi 1 or 2 pattern, or a Hayashi 4 pattern. With a single, clinically explainable exception, no participant had both a Hayashi 1 or 2 pattern and a Hayashi 4 pattern. While this increased variation within the Hayashi patterns suggests that Kraft patterns should be preferred to Hayashi patterns in future research, it must also be noted that Kraft patterns to date, do not have any longitudinal outcome data.

Using insulin resistance measures to assess insulin response patterns

Using the definition of normal insulin tolerance as Kraft I pattern, the McAuley index was unable to distinguish between normal and hyperinsulinaemic sub-groups. This contrasts to the HOMA2 variables and OGIS, which all had clear delineations between the normal and hyperinsulinaemic sub-groups. Returning a similar value across a range of Kraft patterns, HOMA2, and OGIS values suggests the McAuley Index is less sensitive to changes of physical state than the other measures.

Although HOMA2 variants clearly delineated between normal and hyperinsulinaemic states, high variability decreases the sensitivity of the test. Only OGIS had both sensitivity and repeatability. This further questions the value of fasting tests, especially for assessing compensatory hyperinsulinaemia. Our previous research found a poor association between a fasting insulin < 30 μU/mL and a delayed insulin peak (Crofts, Schofield, et al., in submission).

Limitations

We recognise that our study had a number of limitations, especially with respect to participant drop-out rates and small sample size. However, sample sizes of 10 participants are common in repeatability studies for insulin resistance (Gordon et al., 2011; Le, Brookshire, Krakoff, & Bunt, 2009). Nevertheless, this study may be the first to assess the test-retest repeatability of insulin response patterns. Future research for diagnosing insulin resistance should focus on a dynamic test based on an oral glucose tolerance test. There are concerns about using methodologies based on the oral glucose tests due to previous reports of poor repeatability or variable glucose absorption rates. However, our study has shown that dynamic tests have a higher degree of repeatability compared to those based on fasting models. The lower rate of repeatability from models based on fasting tests may be due to the natural lability of insulin, which our study shows has a CV of 25%; a figure consistent with previous reports (Widjaja et al., 1999).

While previous research has focused on diagnosing insulin resistance for the early diagnosis of many metabolic diseases, hyperinsulinaemia is an emerging field (Kelly et al., 2014). Although hyperinsulinaemia is thought to follow insulin resistance, previous research suggests that the two conditions are independent (Kelly et al., 2014). This means it is plausible that hyperinsulinaemia may be corrected while insulin resistance is maintained. Given the high degree of overlap between the conditions, it is also plausible that diagnostic tests for hyperinsulinaemia and insulin resistance may overlap. Furthermore, the mechanisms of hyperinsulinaemia causing metabolic damage are becoming well established, whereas there are no clear mechanistic pathways whereby insulin resistance results in metabolic damage in the absence of hyperinsulinaemia. Given the variability of fasting insulin, dynamic modelling or insulin response patterning may be the most effective way of diagnosing hyperinsulinaemia, and this is where future research should be focused.

Conclusion

Hyperinsulinaemia may indicate metabolic disease earlier than conventional measures but a lack of a consistent testing process hampers ongoing research. As hyperinsulinaemia is closely associated with insulin resistance, assessing the latter may also diagnose hyperinsulinaemia. Fasting insulin resistance measures are not suitable either due to a lack of repeatability (HOMA2 variants) or sensitivity (McAuley Index). Dynamic testing, either using OGIS or insulin response patterns should be further investigated for assessing hyperinsulinaemia but the latter should consider both the magnitude and timing of the insulin peaks.

Chapter 6: Determining a diagnostic algorithm for hyperinsulinaemia

Preface

This thesis has so far established that hyperinsulinaemia is an independent risk factor for metabolic disease (Chapter 2), and can be detected prior to weight gain or hyperglycaemia (Chapter 3). Therefore, the question remains how to best diagnose hyperinsulinaemia. This question was raised in Chapter 2 and has been further investigated in Chapters 3-5. Although many studies have used fasting insulin to define hyperinsulinaemia, this may not be the most suitable method. Chapter 3 showed that during a 100 g oral glucose tolerance test, fasting insulin levels were, for the most part, not associated with the subsequent insulin response pattern.

As hyperinsulinaemia is physiologically associated with insulin resistance (Chapter 2 and depicted in Appendix H) it was hypothesised that measures of insulin resistance, may be able to detect hyperinsulinaemia. HOMA2 represented fasting measures of insulin resistance and OGIS represented dynamic measures. These tests were chosen as they are able to be performed in a community pathology laboratory with no specialised equipment or training.

As fasting insulin is known to be highly variable, Chapter 4 investigated the repeatability characteristics of fasting insulin, HOMA2 and OGIS. HOMA and fasting insulin were shown to be too variable to recommend as reliable clinical diagnostic tests. OGIS was determined to be the best choice for investigating insulin resistance. Chapter 5 further investigated the repeatability characteristics of insulin resistance measures (HOMA2, OGIS and the McAuley Index) and insulin response patterns (Kraft and Hayashi). This chapter also investigated whether insulin resistance measures could predict insulin response patterns. The results reinforced the findings from Chapter 4. Measures based on fasting insulin are unable to predict the insulin response patterns. Kraft patterns were more repeatable compared to Hayashi patterns, suggesting that the magnitude of the insulin response is an important predictor of hyperinsulinaemia.

Overall, the results so far show that hyperinsulinaemia cannot be defined by a fasting measure, but instead by observing the insulin response pattern following a 3-hour oral glucose tolerance test. This make hyperinsulinaemia testing resource intensive, both in

terms of blood analysis and time. This chapter aims to increase accessibility to hyperinsulinaemia testing by determining whether Kraft patterns can be predicted using fewer blood samples and/or other clinical characteristics.

Abstract

Introduction:

Ascertaining Kraft dynamic insulin response patterns following a three-hour 100 g oral glucose tolerance test appears to be the most reliable method for diagnosing hyperinsulinaemia. However, this test may be too resource intense for standard clinical use. This study aims to see if Kraft patterns can be accurately predicted using fewer blood samples with sensitivity/specificity analyses.

Method:

We analysed the results of 4185 men and women with a normal glucose tolerance, who had a 100 g oral glucose tolerance test with Kraft pattern analysis. Participants were dichotomised into normal-low insulin tolerance (Kraft I or V patterns) or hyperinsulinaemia (Kraft IIA-IV patterns). Sensitivity and specificity analysis were applied to available variables (including age, BMI, fasting insulin or glucose) both individually and in combination.

Results:

Out of a maximal combined sensitivity/specificity score of 2.0, two-hour insulin level > 45 μU/mL attained the highest score (1.80). Two-hour insulin alone also attained the highest sensitivity (> 30 μU/mL, 0.98), and the highest specificity (> 50 μU/mL, 0.99) scores. Combining two-hour insulin with other variables reduced the sensitivity and/or specificity.

Conclusion:

People with a two-hour plasma insulin level < 30 μU/mL are unlikely to be hyperinsulinaemic. Given that first line treatment is lifestyle modification, we recommend that a 2-hr plasma insulin level > 30 μU/mL following a 100 g oral glucose tolerance test be used to identify the hyperinsulinaemic individual.

Introduction

A continued rise in obesity is forecast to impose a considerable global burden to health (Wang, McPherson, Marsh, Gortmaker, & Brown, 2011). The prevailing model of obesity suggests an obesogenic environment is the primary driver of obesity, which results in increased metabolic disease (Figure 8**Error! Reference source not found.**). However, there is growing interest in the metabolic theory of obesity. This theory suggests that obesity primarily results from a metabolic disorder (Ludwig & Friedman, 2014). Implicit in this model is that hyperinsulinaemia is a key driver of the obesity process, potentially as a result of dietary, genetic and other lifestyle factors. It is well-recognised that, amongst other actions, insulin suppresses lipolysis, regulates cellular glucose uptake and is considered to be an anabolic hormone thereby being a key driver of weight gain.

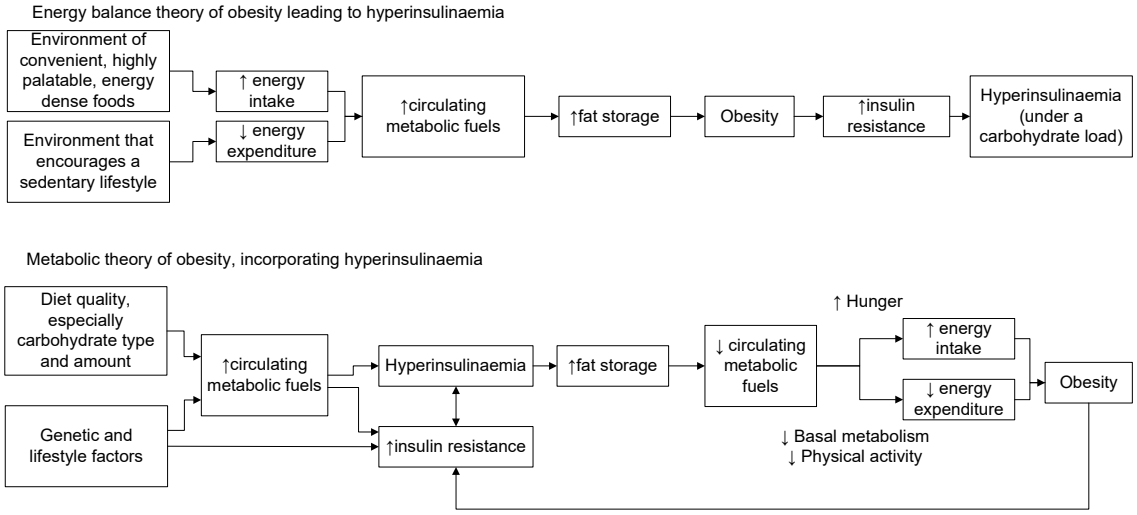

Figure 8: Traditional and metabolic theories of obesity and metabolic disease (adapted from Ludwig & Friedman, 2014).

Obesity is not the only reason to be concerned about hyperinsulinaemia. Hyperinsulinaemia also contributes to metabolic disease via inflammatory pathways, by increasing cellular growth and proliferation via IGF-1, and being proatherosclerotic via decreased nitric oxide production, impaired fibrinolysis and increasing triglyceride production (Olefsky et al., 1974; Pollak, 2008; Rask-Madsen & King, 2007; Stegenga et al., 2006). The potential prevalence of hyperinsulinaemia is concerning. Our previous work showed that not only should all people with impaired glucose tolerance or type 2 diabetes be considered hyperinsulinaemic by default, but a substantial proportion of the population with normal glucose tolerance are also at risk of hyperinsulinaemia (Crofts, Schofield, et al., in submission). This suggests that early detection of

hyperinsulinaemia may aid public health initiatives as this condition is suspected to precede other metabolic changes such as hypertension or dyslipidaemia.

Diagnosing hyperinsulinaemia is problematic as there are no agreed reference ranges. Furthermore, most of the previous insulin-related research is in the field of insulin resistance, which, although intertwined with hyperinsulinaemia, is an intrinsically different condition. Insulin resistance can simply be defined as "the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilisation in an individual as much as it does in the general population" (Lebovitz, 2000). The gold-standard for measuring insulin resistance is the hyperinsulinaemic-euglycaemic clamp. During this test, insulin is infused into the person at supra-physiological concentrations, while sufficient glucose is simultaneously administered to maintain euglycaemia. As this combination has the effect of preventing gluconeogenesis, once the person reaches steady-state, the glucose infusion rate equals the body-wide rate of glucose uptake. This is considered to be the measure of cellular sensitivity to insulin. The hyperinsulinaemic-euglycaemic clamp test though, cannot determine if the person is hyperinsulinaemic under normal physiological conditions.

Under normal physiological conditions, a person can only become hyperinsulinaemic when two conditions are met. The first, is when a person has a degree of insulin resistance, which may occur acutely, or chronically. Acute insulin resistance can occur under conditions of hypoglycaemia or high cortisol levels, when glucose needs to be preferentially shunted to the brain. Chronic insulin resistance may occur for a variety of reasons, including chronic stress, elevated free-fatty acids, certain medications, and hyperinsulinaemia. The second is under conditions of a carbohydrate load. Insulin is predominantly released from the pancreas in response to elevated blood glucose levels. This means that a person can be chronically insulin resistant, but not become hyperinsulinaemic if they restrict their dietary carbohydrate intake. This phenomenon may be one reason why insulin resistance testing has not been shown to improve disease risk calculations.

There may be other reasons why the epidemiology fails to support the notion that insulin resistance precedes obesity. Hyperinsulinaemia is unlikely to be the sole cause of obesity and metabolic disease. Changes to the built environment, genetics, the impact of foods with low nutrient density and changes to the gut microbiome are all believed to impact obesity but may or may not directly affect insulin sensitivity.

Furthermore, the methods used to assess insulin resistance can be problematic. The hyperinsulinaemic-euglycaemic clamp test is unsuitable for wide-scale epidemiological studies. Simpler tests, such as the homeostasis model assessment (HOMA) were developed so that the effects of the clamp test could be modelled based on a fasting insulin and glucose blood test. However, although it was assumed that fasting insulin levels could be used to assess insulin resistance, or even hyperinsulinaemia, this has not been shown in practice. People with a fasting plasma insulin ≤ 30 μU/mL can have markedly disparate plasma insulin responses following an oral glucose tolerance test (Crofts, Schofield, et al., in submission). This phenomenon may be partially explained by the pulsatile secretory nature of insulin which has been shown to have a coefficient of variation from 25-50% (Mather et al., 2001). This means that fasting insulin is insufficiently reliable for clinical purposes.

Therefore, in order to effectively understand hyperinsulinaemia, a new method for diagnosis and monitoring needs to be developed. The most promising research has been based around insulin response patterns, formed during an oral glucose tolerance test. Kraft (1975) demonstrated five distinct insulin response patterns arising during a three-hour 100 g oral glucose tolerance test. These patterns were based on both the magnitude and timing of the insulin peak, and the rate of decay of the response. Using Kraft's definitions (1975), a normal insulin response is considered to be a fasting insulin < 30 μU/mL along with an insulin peak at 30 or 60 minutes, followed by a rapid rate of decay such that the sum of the 2-hr plus 3-hr insulin concentration is < 60 μU/mL. A hyperinsulinaemic response occurs with any combination of: raised fasting insulin; a delayed insulin peak at 2-hrs or later; or a slow rate of decay. A hypoinsulinaemic response occurs when every plasma insulin value is ≤ 30 μU/mL. Our previous work examined the Kraft database and simplified the original algorithm (Table 18).

Hayashi and colleagues used different insulin response patterns. They measured plasma insulin at baseline and then at 30, 60, and 120 minutes during a two-hour, 75 g oral glucose tolerance test. By determining the timing of the insulin peak/s, as assessed by the responses, they showed an increased risk of developing type 2 diabetes in people who had an insulin response that peaked at two hours compared to those who had an insulin peak at 30 or 60 minutes (Hayashi et al., 2013).

Table 18: Kraft pattern algorithm

Kraft Pattern	Description
Pattern I (Normal insulin)	• Fasting insulin ≤ 30 µU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum < 60 µU/mL
Pattern IIA (Borderline)	• Fasting insulin ≤ 50 µU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum ≥ 60, < 100 µU/mL OR • Fasting insulin 31-50 µU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum < 60 µU/mL
Pattern IIB (Hyperinsulinaemia)	• Fasting insulin ≤ 50 µU/mL • 30 min or 1-hour peak • 2-hour + 3-hour sum ≥ 100 µU/mL
Pattern III (Hyperinsulinaemia)	• Fasting insulin ≤ 50 µU/mL • Delayed peak (2-hour or 3-hour)
Pattern IV (Hyperinsulinaemia)	• Fasting insulin > 50 µU/mL
Pattern V (Hypoinsulinaemia)	• All values ≤ 30 µU/mL

Our previous research suggests that Kraft patterns should be preferred to the Hayashi patterns as Kraft patterns demonstrated less variability (Crofts, Wheldon, Zinn, & Schofield, in draft). The disadvantage to using insulin response patterns is the sheer number of blood tests that are required. Kraft patterns require five blood samples taken over three hours, while Hayashi patterns are based on four blood tests taken over two hours.

It is also plausible that other clinical features influence, or are influenced by, hyperinsulinaemia. For example, Hayashi and colleagues demonstrated that different glucose response patterns were produced depending on the patient's insulin response curve (2013). Therefore, it is plausible that we can predict a patient's insulin response pattern by a clinical profile instead.

Sensitivity and specificity analyses are statistical binary classification measures used to assess the proportions of correctly diagnosed people suspected of having a clinical

diagnosis. Sensitivity measures the proportion of correctly identified people with the clinical condition (sick), while specificity measures the proportion of correctly identified people without the clinical condition (healthy) as according to the methods of Altman and Bland (1994). Ideally a test should have a combined sensitivity and specificity sum of close to 2.0 as possible. In practice this is less likely to occur, and it must be decided whether to focus on sensitivity or specificity. When sensitivity is maximised, at the expense of specificity, it means that sick people are less likely to be misdiagnosed as healthy, but the proportion of false negatives, i.e., when healthy people are misdiagnosed as being sick, is increased. This option should be preferred when the risk associated with missing people is high (e.g. an infectious epidemic) and/or the first line treatment is of low risk (e.g. lifestyle measures). The reverse occurs when specificity is maximised. This study will use a variety of clinical features gathered during a three-hour 100 g oral glucose tolerance test and apply sensitivity and specificity analyses to determine whether the insulin response pattern can be accurately predicted.

Method

Participants: 15,000 patients and healthy volunteers were referred for an oral glucose tolerance test at St Joseph Hospital, Chicago. IL. U.S.A. between 1972 and 1992. Data collected included plasma glucose, plasma insulin, age, gender, height, and weight.

Reanalysis inclusion:
From this database, we included 2161 men aged older than 20 years, and 2024 women aged greater than 45 years who had a normal glucose tolerance as defined by WHO criteria (1999) and also had age, height and weight recorded; a total of 4185 participants (Table 19).

Reanalysis exclusion:

Exclusion criteria included a BMI $\leq$ 17.9 kg/m^2 due to the potential confounder of concurrent illness. Women aged between 20-45 years were excluded due to the potential confounder of pregnancy.

Table 19: Participant characteristics

	Total
n	4185
female	2024 (48)
Age (years)	
male	44.9 (15.2)
female	59.1 (9.4)
BMI (kg/m^2)	25.9 (4.7)
Plasma insulin (μU/mL)	
0 min	13 (13)
30 min	87 (56)
60 min	105 (73)
120 min	77 (62)
180 min	40 (41)
Plasma glucose (mg/dL)	
0 min	86 (10)
30 min	152 (32)
60 min	146 (43)
120 min	101 (22)
180 min	82 (25)

Frequency data are reported as n (%), otherwise mean (SD)

Study Protocol

Subjects fasted overnight for 10-16 hours. A fasting venous blood sample was taken; 100 g of glucose (Glucola, Miles/Ames, Elkhardt, IN.) was ingested and venous samples at 30 minutes, 60 minutes, and each subsequent hour for between three and five hours. The blood specimens were measured for glucose and insulin. Originally the ferricyanide method (Autoanalyzer, Technicon Corporation) was used to analyse glucose, but this was later changed to plasma glucose oxidase method (Autoanalyzer, Technicon Corporation, Tarrytown, N.J., Vitros, Johnson and Johnson Clinical Diagnostics, Inc., Rochester, N.Y.). Glucose samples analysed with the ferricyanide method were adjusted downward by 10 mg/dL to account for the systematic error according the methods of Passey and colleagues (1977).

Plasma insulin was determined from the samples stored at -70°C by a commercial double-antibody solid phase radioimmunoassay, (Pharmacia insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden). The Phadebus Insulin Test had duplicate procedure precision of one standard deviation = ± 5 microunits in measurements up to 150 microunits.

Ethics

This study was granted ethical approval by Health and Disability Ethics Committee (New Zealand) on 30 October 2013. Approval reference: 13/CEN/166. AUTEC reference: 13/337.

Analysis

This study uses current clinical practices and sensitivity and specificity calculations to logically derive whether Kraft's patterns can be simplified.

Normal insulin response

Sensitivity and specificity calculations can only be performed with a dichotomised test outcome. Therefore, this study separates the Kraft patterns into low-to-normal insulin responses (Kraft I, V) and hyperinsulinaemic responses (Kraft IIa-IV) as per the algorithm listed in Table 18. People with a fasting insulin > 30 μU/mL are automatically defined as hyperinsulinaemic using Kraft's definitions (2011, p. 29). Sensitivity and specificity calculations were performed as according to the methods of Altman and Bland (1994).

Variables

The variables to be tested individually and in combination within the sensitivity and specificity calculations included body mass index (BMI), age, HOMA 2%B, HOMA 2%S, HOMA 2IR, oral glucose insulin sensitivity (OGIS), and plasma glucose or insulin levels from each time point (0 min, 30 min, 1-hr, 2hr, and 3hr). HOMA 2 variables and OGIS were calculated using their respective calculators (Diabetes Trials Unit, 2004; Mari, n.d.).

Results

As shown in Table 20, a 2-hr insulin level > 30 μU/mL attained the highest sensitivity (0.98), a moderate specificity (0.62) and an overall score of 1.6 from a possible 2.0. This means that in a sample of 100 people with hyperinsulinaemia and 100 people with normal insulin tolerance, this test would correctly identify 99 of the people with hyperinsulinaemia as being hyperinsulinaemic. However, of the 100 people with normal insulin tolerance, 38 people would be identified as being hyperinsulinaemic, when in fact they have a normal insulin tolerance.

The highest overall score was 2-hr insulin > 45 μU/mL (1.80) and the highest specificity was 2-hr insulin > 50 μU/mL (0.99). The 2-hr insulin alone achieved high scores for sensitivity, but this score dropped if applied in combination with another variable such as glucose. For example, 2-hr glucose > 80 mg/dL achieved scores of 0.9, 0.38, and 1.28 for sensitivity, specificity and the total sum respectively. 2-hr insulin > 45 μU/mL achieved scores of 0.85, 0.95 and 1.8 for sensitivity, specificity and the total sum respectively. However, the combination of 2-hr glucose > 80 mg/dL and 2-hr insulin > 45 μU/mL only attained a score of 0.78 for sensitivity, 0.96 for specificity and a combined result of 1.74. Although this is still a very good score, the sensitivity is lower than using 2-hr insulin in isolation.

OGIS < 600 $mL.min^{-1}.m^{-2}$ attained the highest score (1.30) of the measures for insulin resistance with a very high sensitivity score (0.95). HOMA 2 variables did not score highly overall: HOMA2 %B > 20 scored 1.27 while HOMA 2 IR > 0.2 scored 1.32.

Table 20: Sensitivity and specificity calculations

Test variable	Sensitivity	Specificity	Sum SS
2-hr insulin > 30 μU/mL	0.98	0.62	1.60
OGIS < 600 mL.min^{-1}.m^{-2}	0.95	0.34	1.30
2-hr insulin - fasting insulin > 30 μU/mL	0.90	0.83	1.73
2-hr glucose > 80 mg/dL	0.90	0.38	1.28
HOMA2 %B > 20	0.87	0.40	1.27
1-hr insulin > 50 μU/mL	0.86	0.49	1.36
2-hr insulin > 45 μU/mL	0.85	0.95	1.80
Age > 35 years	0.85	0.24	1.09
2-hr insulin - fasting insulin > 35 μU/mL	0.84	0.92	1.76
2-hr glucose - fasting glucose > 0 mg/dL	0.83	0.47	1.31
fasting insulin > 5 μU/mL	0.83	0.46	1.29
1-hr insulin > 60 μU/mL	0.80	0.61	1.40
2-hr insulin > 50 μU/mL	0.79	0.99	1.78
3-hr insulin > 20 μU/mL	0.79	0.85	1.64
2-hr insulin > 45 μU/mL and 2-hr glucose > 80 mg/dL	0.78	0.96	1.74
OGIS < 500 mL.min^{-1}.m^{-2}	0.70	0.84	1.54
2-hr insulin > 45 and 2-hr glucose > 90	0.69	0.97	1.67
2-hr glucose-fasting glucose > 10 mg/dL	0.68	0.67	1.35
2-hr insulin-fasting insulin > 50 μU/mL	0.65	1.00	1.64
2-hr glucose > 100 mg/dL	0.63	0.73	1.35
Age > 50 years	0.61	0.52	1.13
3-hr insulin > 30 μU/mL	0.60	0.99	1.58
fasting glucose > 85 mg/dL	0.56	0.46	1.02
2-hr insulin > 45 μU/mL and 2-hr glucose > 100 mg/dL	0.55	0.98	1.54
BMI > 25 kg/m^2	0.55	0.61	1.16
BMI > 25kg/m^2, 2-hr insulin > 30 μU/mL	0.54	0.83	1.37
fasting insulin > 10 μU/mL	0.54	0.79	1.32
HOMA2 IR > 0.2	0.52	0.81	1.32
2-hr glucose - fasting glucose > 20 mg/dL	0.50	0.81	1.31
Age > 35 years and BMI > 25 kg/m^2	0.48	0.70	1.17
BMI > 30 kg/m^2	0.16	0.91	1.07
2-hr insulin > 20 μU/mL	0.12	0.99	1.11
fasting glucose > 80 mg/dL and fasting insulin > 20 μU/mL	0.09	0.99	1.08

Discussion

This study aimed to determine if there was a simple test that could diagnose hyperinsulinaemia, as defined by Kraft patterns IIa, IIb, III and IV. We were looking for a test with a high degree of sensitivity but required the least amount of resources, including time. We found that a 2-hr plasma insulin level > 30 μU/mL following a 100 g, 2-hr OGTT provided the highest degree of sensitivity in predicting a hyperinsulinaemic pattern.

Although other variable combinations attained a higher combined sensitivity and specificity score, there are a number of reasons why we believe that a 2-hr plasma insulin cut-off of 30 μU/mL is the most useful test to recommend for both clinical and research practice. Two-hour plasma insulin alone featured prominently in the calculations with different levels attaining the highest sensitivity, specificity and combined score. Furthermore, using a 2-hr level aligns with current OGTT protocols for diabetes diagnosis and a single insulin level is relatively inexpensive to analyse compared with other potential test methodologies including OGIS.

Additionally, using the lowest 2-hr insulin level that maintained a reasonable sensitivity and specificity seemed the most appropriate clinical decision. Although a 2-hr level > 45 μU/mL attained the highest summed score of 1.8, it had a lower sensitivity of 0.85 compared with 0.98 for a 30 μU/mL cut off. A sensitivity score of 1.0 means that everybody who is tested for the disease, who truly has the disease will be given a correct diagnosis. When sensitivity scores are decreased to 0.85, this means 15% of people who truly have the disease will be told, falsely, that they have a negative result. A lower specificity score increases the possibility of false negative results, or when people will be told that they have the disease, when they are, in fact, disease free.

The decision to err on the side of sensitivity or specificity also depends on the available management strategies should a diagnosis be made. If the potential treatment is associated with significant risks relative to benefits, then the decision may be based on specificity. For hyperinsulinaemia, the potential first-line treatments include physical activity (DiPietro, Dziura, Yeckel, & Neufer, 2006) and dietary strategies (Ryan et al., 2013; Shai et al., 2008). Given that the risks associated with treatment are low when compared to potential benefits, we have erred on the side of sensitivity.

One criticism of using insulin is that 2-hr levels have a high degree of variability. Our previous study showed that the repeatability coefficient of 2-hr plasma insulin following a 100 g OGTT was approximately 45 μU/mL (282 pmol/L) (Crofts et al., in draft). Given the limits of sensitivity and specificity ranged between 30 and 50 μU/mL, we believed that the variation as shown by the repeatability coefficient would not have a significant impact on clinical outcome, but further research is needed to confirm.

Fasting insulin levels ≤ 30 μU/mL were not useful in determining hyperinsulinaemia. Levels at the lowest end of the current reference range had a high sensitivity, but low specificity. We agree with current recommendations that neither hyperinsulinaemia nor insulin resistance should be diagnosed on the basis of a fasting insulin test (Samaras et al., 2006). It is debatable as to whether fasting insulin levels are useful in the overall diagnostic process for hyperinsulinaemia. In our study, fasting insulin levels only detected hyperinsulinaemia in 238 of the 3409 (7.0%) people with hyperinsulinaemia (Figure 9).

We were disappointed that we could not recommend a fasting test for hyperinsulinaemia. However, variables such as BMI, fasting glucose and fasting insulin, either alone or in combination did not attain sufficient sensitivity or specificity. However, our database did not capture additional information, such as ethnicity, or other potential prognostic markers such as uric acid or liver enzymes (R. J. Johnson et al., 2009; Wannamethee, Shaper, Lennon, & Whincup, 2005). These markers are linked to metabolic syndrome, and as such may also be linked to hyperinsulinaemia.

A significant limitation to our study is the lack of long-term health outcomes due to the cross-sectional nature of the Kraft database. We cannot at this stage evaluate the effectiveness of this test in actually predicting the risk of future disease. Previous work has shown that elevated 2-hr insulin levels are associated with increased risk of developing type 2 diabetes (Hayashi et al., 2013), therefore our conclusions are plausible. However, either new prospective studies, or reanalysis of studies that have collected both the 2-hr insulin level and long-term outcomes are required.

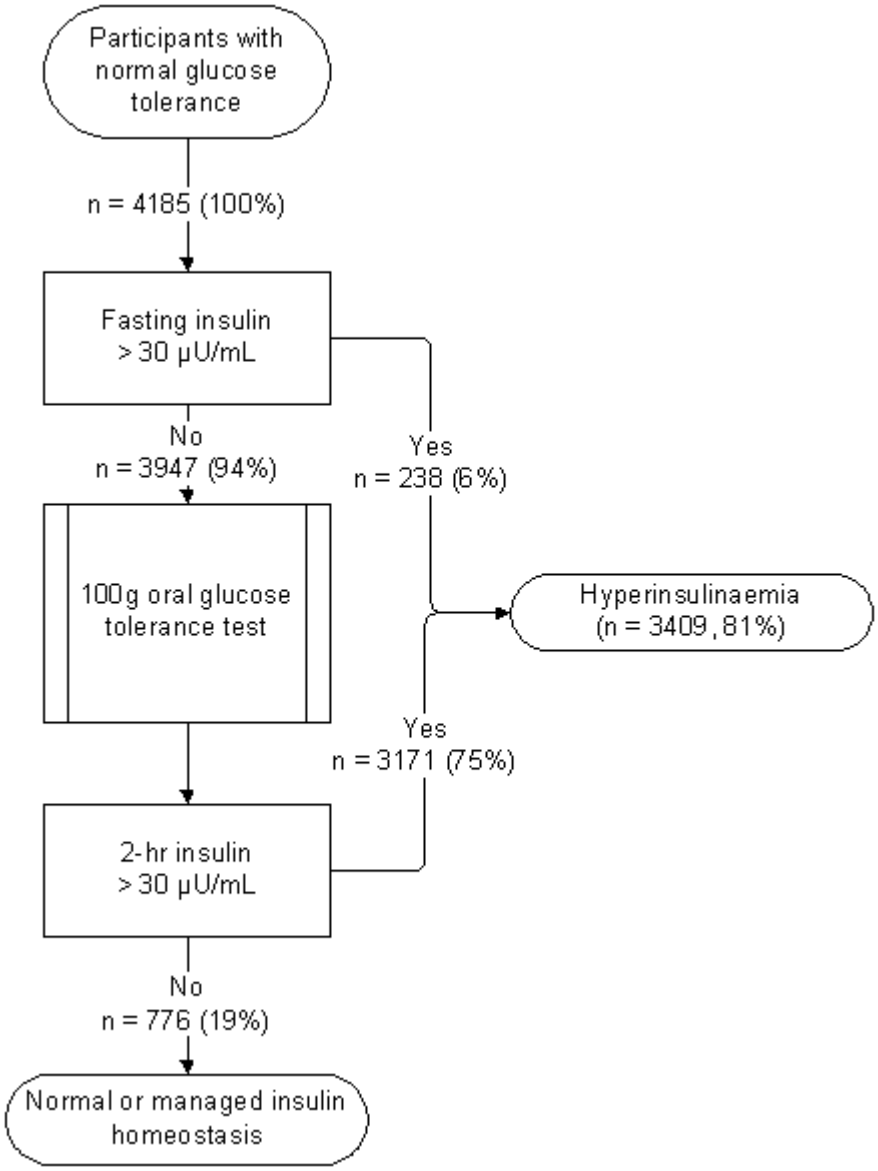

Figure 9: Diagnostic algorithm for hyperinsulinaemia

Conclusion

Hyperinsulinaemia is conclusively linked with many metabolic diseases (Kelly et al., 2014), but this disease may be silent and not be associated with obesity (Crofts, Schofield, et al., in submission). Identifying the normoglycaemic individual with concurrent hyperinsulinaemia may benefit public health initiatives. We recommend that a 2-hr plasma insulin level > 30 µU/mL following a 100 g oral glucose tolerance test be used to identify the hyperinsulinaemic individual.

Chapter 7: Hyperinsulinaemia: Best management practice.

Preface

This thesis has so far shown that hyperinsulinaemia is an independent long-term risk factor for metabolic disease (Chapter 2). Hyperinsulinaemia can be accurately diagnosed using a 2-hr plasma insulin level following a 100 g oral glucose load (Chapter 3). The question then remains "so what?" Ethically, it is inappropriate to diagnose a pathology without having potentially beneficial management strategies. Hyperinsulinaemia is under-recognised and few studies have investigated means of management. Given early management of hyperinsulinaemia may be a viable means of reducing the prevalence of many metabolic diseases, investigating management strategies is urgently needed. However, many clinicians and/or their patients may be unwilling to wait until research outcomes are translated into practice. Therefore, this chapter is intended as a theoretical guide for clinicians and patients for managing hyperinsulinaemia, focusing on clinical, pathological and epidemiological research.

Abstract

Chronic hyperinsulinaemia associated with insulin resistance is directly and indirectly associated with many metabolic disorders that contribute to significant morbidity and mortality. Because hyperinsulinaemia is not widely recognised as an independent health risk, there are few studies that assess management strategies. Medication management may not address the multiple issues associated with hyperinsulinaemia. Lifestyle management includes physical activity, especially high intensity interval training, and dietary management. Reducing carbohydrate quantity and increasing nutrient density by improving carbohydrate quality are discussed as treatment strategies for the hyperinsulinaemic individual.

Physical activity and dietary management provide the foundation for hyperinsulinaemia management and may work synergistically. Of these principles, a combination of resistance and high intensity interval training, and carbohydrate restriction are the two most effective frontline management strategies for managing hyperinsulinaemia.

Overview

Compensatory hyperinsulinaemia (further referred to as "hyperinsulinaemia") is associated, mechanistically and epidemiologically, with many chronic metabolic diseases (Crofts et al., 2015; Kelly et al., 2014). The aetiology of hyperinsulinaemia is likely heterogeneous (Crofts et al., 2015) and in the earliest stages asymptomatic (Crofts, Schofield, et al., in submission). Early management of hyperinsulinaemia may prevent, delay, or mitigate the severity of subsequent pathologies. Although hyperinsulinaemia is a common co-pathology with impaired glycaemic control, this paper focuses on the management of compensatory hyperinsulinaemia in the presence of normal glucose tolerance.

There are several different states that depict the continuum that reflects healthy insulin response through to hyperinsulinaemia and finally, impaired glycaemic control as described in Chapter 2. It is proposed that people transition between different states, which may be either acute or chronic, depending on the circumstances at the time, and may be subject to change (Appendix H). The close relationship between the two different states of hyperinsulinaemia and insulin resistance can also be noted. This means that as well as targeting insulin levels directly, strategies that improve insulin sensitivity, especially the up-regulation of GLUT4, will also reduce hyperinsulinaemia. As there are few studies that directly assess hyperinsulinaemia management strategies, this review will include strategies that improve glycaemic control in the absence of evidence of increased insulin secretion. It will also consider strategies that provide symptomatic improvement of conditions associated with hyperinsulinaemia such as polycystic ovarian syndrome (PCOS).

There are two main strategies for managing hyperinsulinaemia: maximising insulin sensitivity and reducing glycaemic load. Insulin sensitivity can be maximised via up-regulating glucose transporter type 4 (GLUT4) or insulin receptors, or by preventing (further) insulin resistance. Glycaemic load may occur through two main pathways, endogenous through metabolic pathways such as gluconeogenesis, glycolysis, or renal reabsorption (Triplitt, 2012), and exogenous via dietary intake.

There are three main mechanisms to achieve each of these strategies: Physical activity, diet, and medicines and other supplements.

Sources and selection criteria

Literature was reviewed on hyperinsulinaemia and insulin resistance, targeting full-text English language studies. There was no date criterion. Articles were selected on the basis of having a minimum of both a plausible biological mechanism and established clinical association. An academic database search included EBSCO, Medline and Google Scholar, using variants of the terms "hyperinsulinaemia," "insulin resistance," "type 2 diabetes," and "metabolic syndrome," and each of these terms in conjunction with variants of "diet," "nutrition," "physical activity," "pharmacology," and "treatment." References were based on the authors' judgment of relevance, completeness, and compatibility with clinical, epidemiological, pathological and biochemical criteria.

Physical activity

Physical activity is well-documented for improving insulin sensitivity. Mechanistically this occurs via GLUT4 up-regulation, increased hexokinase gene transcription (Holloszy, 2005), increased fuel consumption and, if sustained, decreases to insulin secretion (Sigal, Kenny, Wasserman, & Castaneda-Sceppa, 2004). Conversely, sustained physical activity can also increase glucagon, cortisol and catecholamine secretion (Sigal et al., 2004). These hormones can all increase gluconeogenesis and if unbalanced, aggravate rather than improve insulin sensitivity. Very intense physical activity stimulates insulin production, especially in the presence of hyperglycaemia. Without question, physical activity will be a key component for managing hyperinsulinaemia, but the question remains whether different forms of physical activity can maximise sensitivity while minimising counter-hormones.

Physical activity can be broadly divided into two main classifications that have considerable overlap: resistance training and aerobic activity. The latter has a further subset: high intensity interval training (HIIT).

Resistance training

Resistance training is characterised by muscles contracting against an external resistance causing brief and isolated activity of single muscle groups (Yang, Scott, Mao, Tang, & Farmer, 2014). The health-benefits of resistance training are well-recognised. These can include decreases to HbA1c, weight, body fat, and blood pressure (Westcott, 2012). Other improvements include increases to bone mineral density, and lean body

mass. There are also potential benefits to mood and cognition, balance and falls-risk, and overall self-esteem.

Resistance training may improve hyperinsulinaemia through three main mechanisms: increasing, or maintaining muscle mass, glucose expenditure and enhancing the cellular metabolic capacity. It is estimated that inactive adults lose 3-8% of muscle mass per decade accompanied by a reduction in resting metabolic rate (Westcott, 2012). Losing muscle mass means that glucose disposal will be harder resulting in increased adiposity. Increased muscle mass is posited as one explanation for the improvements in glucose disposal rates for resistance training (Roberts, Little, & Thyfault, 2013). This is because both weight lifters and long-distance runners show increased glucose disposal rates compared to controls; however, this difference remains only for the long-distance runners after differences in lean-body-mass are taken into account. This is consistent with other studies comparing aerobic to resistance training, which only showed improvements in glucose disposal when the results were expressed per kilo of fat-free-mass.

While resistance training is believed to enhance cellular metabolic capacity by mechanisms such as GLUT4 mobilisation (Roberts, Little, et al., 2013), potentially negative effects by way of increased cortisol are also observed. Crucially, fewer repetitions and longer rest periods between sets elicit a lower cortisol response, which may be important for beginners to resistance training (Kraemer & Ratamess, 2005). Increased catecholamine and/or insulin secretion may also be observed with resistance training. These changes may also be exercise-dose dependent and may attenuate as training adaptation occurs. An elevated insulin response is associated with protein/carbohydrate supplementation. Elevated hormonal responses may also be associated with overtraining (Kraemer & Ratamess, 2005).

Aerobic exercise

Aerobic exercise can be broadly described as light to moderate intensity activities that can be performed for extended periods of time. Examples of aerobic exercise include walking, jogging and swimming. There is a large body of literature on the type and amount of aerobic activity required to maintain health. Conventional wisdom suggests that a minimum of 30 accumulated minutes of moderate intensity activity (i.e., brisk walking) should occur on most days to achieve health benefits (Blair, Kohl, Gordon, & Paffenbarger, 1992), although the efficacy of this volume has since been questioned

(Blair, LaMonte, & Nichaman, 2004). Aerobic exercise is believed to improve metabolic health via the same mechanisms as resistance training.

A meta-analysis comparing resistance training to aerobic exercise concluded that clinically, there were no advantages between resistance training and aerobic exercise for lowering HbA1c or impacting cardiovascular risk (Yang et al., 2014). However, aerobic exercise was modestly advantageous for lowering BMI. Resistance training may confer greater benefit to those with limited mobility as many of the exercises can be performed by the sedentary.

High intensity interval training (HIIT)

HIIT protocols are a subset of aerobic exercise characterised by short, maximal-intensity, anaerobic exercise sessions separated by medium or low intensity periods for recovery. There are several advantages to HIIT protocols compared to conventional aerobic exercise: time; glucose utilisation and cellular metabolic capacity. Lack of time is the biggest reason cited for not exercising (Roberts, Little, et al., 2013). HIIT protocols allow for greater power output for an equivalent amount of energy expenditure but in a shorter period of time (Cockcroft et al., 2015) resulting in greater improvements to cardiorespiratory fitness (Cornish, Broadbent, & Cheema, 2011). Other benefits of HIIT compared to conventional aerobic training include greater reductions of skin-fold thickness and decreased $AUC_{insulin}$ (Roberts, Hevener, & Barnard, 2013; Tremblay, Simoneau, & Bouchard, 1994). HIIT protocols may have further advantages over traditional aerobic exercise regimes as they can be used safely and effectively in people following cardiac stenting, coronary artery grafting and myocardial infarction. Musculoskeletal injuries were no more common than that found with other forms of exercise (Cornish et al., 2011; Gillen et al., 2012; O'Keefe et al., 2012; Shiraev & Barclay, 2012). These results demonstrate that HIIT is safe and effective when performed under controlled conditions. Patients new to HIIT may require specific assessment and/or instructions from an exercise physiologist or physiotherapist.

Summary

GLUT4 adaptation can occur with single bouts of exercise and effects persist for up to 40 hours (Metcalfe et al., 2015; Schnurr et al., 2015). This suggests that, especially in the early days of adopting physical activity, varying the activities undertaken may maximize GLUT4 adaptation while minimising effects from over-secretion of cortisol

or glucagon. While the literature suggests the ideal activity should comprise a combination of resistance training and HITT protocols, the final selection of physical activities may be influenced by personal circumstances, including preference, health status and levels of training required.

Diet

There is considerable public and scientific debate and discussion concerning the optimal dietary approach for the management of metabolic dysregulation. Without discussing macronutrient proportions, it is generally agreed that a healthy diet should predominantly be comprised of the following:

1. Whole foods (Jacobs & Tapsell, 2007)
2. Adequate protein and other currently established essential nutrients including water, specific vitamins, minerals, electrolytes and fatty acids (Westman, 2002).
3. Adequate energy.
4. Adequate fibre. Although fibre may not be considered essential, there is sufficient evidence to support its inclusion (McAuley et al., 2006; Ministry of Health, 2015; Tonstad, Malik, & Haddad, 2013).

A diet that limits the risk of, or manages the effects of, hyperinsulinaemia should also consider the following:

5. Prevents acute hyperglycaemia, whether via either exogenous carbohydrate or gluconeogenesis, thus preventing acute hyperinsulinaemia.
6. Prevents caloric overload, thus limiting both the amount of energy to be stored as fat and the potential for hyperglycaemia.
7. Limits items known to down-regulate GLUT4 or insulin receptors (e.g. arachidonic acid).
8. Promotes items known to up-regulate GLUT4 or insulin receptors.
9. Causes sufficient satiety so that hormones, receptors and transporters are not over-stimulated.

Adherence factors, including adverse reactions should also be considerations. Adherence is recognised as being key to weight-loss (Pagoto & Appelhans, 2013). Traditionally, obesity is seen as the driver of many metabolic diseases, so weight-loss is the first step to improved health (Ludwig & Friedman, 2014). However, the metabolic

theory of disease states that metabolic changes including hyperinsulinaemia may precede weight gain. Under this model, weight-gain is the first visible symptom of metabolic disease, therefore weight-loss should also indicate health improvements. This means that dietary adherence will also be associated with improvements to hyperinsulinaemia.

This research is complicated as many studies use "standard" diets as the control. This "standard" diet is generally low in fruits and vegetables and high in sugars and refined carbohydrates (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 1980). As this diet will likely be lacking in essential nutrients and fibre, cause acute hyperglycaemia, and have excessive calories, any dietary regime that reverses these trends will show improvements to health. Furthermore, diet-health research often employs weight loss as the primary end-point; rather than other metabolic markers, yet improvements to metabolic markers are possible without significant weight changes (Kraft, 1975). However, any dietary approach that causes weight loss, will improve hyperinsulinaemia as body fat can only be stored, rather than oxidised in the presence of high insulin levels (Kovacs & Ojeda, 2012). Therefore, both improved glycaemic control and weight loss can be used as proxies for improved hyperinsulinaemia.

There are three distinct dietary approaches (low fat; Mediterranean; and carbohydrate-restricted) that are shown to improve diabetes by improving glycaemic control. Improved glycaemic control may indicate improved insulin response, so these diets should be considered for managing hyperinsulinaemia. Although there is some evidence to support high protein diets for the treatment of diabetes, excess protein will induce gluconeogenesis, thus breaching criterion 4. Therefore, only moderate protein diets will be considered in this review. As few studies directly target hyperinsulinaemia, the question remains are any of these three approaches superior to the others for managing hyperinsulinaemia?

Low-fat

Currently, the low fat, high carbohydrate dietary approach is considered to be standard practice for managing diabetes by many authorities. For adults (aged 19 and older) this regime generally comprises 20-35% fat, (< 10% saturated fats), 10-35% protein and 45-65% carbohydrate (U.S. Department of Agriculture & U.S. Department of Health

and Human Services, 2010). Fruits, vegetables and whole-grains are recommended as carbohydrate and fibre sources, while vegetable oils (excluding coconut, palm and palm kernel oils) are emphasised as healthy fat sources (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 1980). Lean protein, including fat-free or low-fat dairy products, or vegetable protein sources, are also recommended.

Mediterranean

Although there are a variety of "Mediterranean" dietary approaches, (Noah & Truswell, 2001), the term generally defines a diet that comprises a high amount of monounsaturated fatty acids (MUFA), predominantly from olive oil (35%), fruits and vegetables, whole-grains and fish; moderate amounts of alcohol and small amounts of red meat, sugars and refined grains (Nordmann et al., 2011; Willett & Skerrett, 2001).

Carbohydrate-restriction

Like the Mediterranean diet, there is no clear definition of a carbohydrate-restricted diet. Daily carbohydrate intake has been defined as 12 - 40% of daily energy intake or < 20 - 150 g/day (Gardner et al., 2007; Johnstone, Horgan, Murison, Bremner, & Lobley, 2008; Shai et al., 2008; Volek & Phinney, 2013). To ensure adequate energy, the fat content of the diet is increased, up to about 75% of daily energy content.

Comparison of different dietary strategies

Each of these diets have notable benefits for the management of diabetes compared to standard diets (Feinman et al., 2015; Pérez-López, Fernández-Alonso, Chedraui, & Simoncini, 2013; Salas-Salvadó, Martinez-González, Bulló, & Ros, 2011). It is traditionally considered that weight management is the key driver behind metabolic improvements, hence the previous favour of the low-fat (and consequently low-calorie) diet. However, emerging research suggests that increased benefits to metabolic health can be found from diets higher in fats and lower in carbohydrates. A meta-analysis compared Mediterranean diets to low-fat diets in overweight/obese people (n = 2650, 50% female) over two years of follow-up. Those following the Mediterranean diet had greater improvements to body weight and BMI, systolic and diastolic blood pressure, fasting glucose, total cholesterol, and high-sensitivity C-reactive protein (hs-CRP) (Nordmann et al., 2011). While some of the effects were modest, the weighted mean differences clearly favoured the Mediterranean diet. This suggests that low-fat diets may not be optimal for managing diabetes, or hyperinsulinaemia.

Although this study does not directly assess hyperinsulinaemia, the improvements to the other metabolic markers, especially fasting glucose, imply improvements to hyperinsulinaemia. There are several potential mechanisms for these observations. Firstly, the lower carbohydrate content and therefore glycaemic load means that acute hyperglycaemia, and hence acute hyperinsulinaemia is less likely (Rossi et al., 2013). Fewer glucose molecules to be absorbed into the cells reduces metabolic stress. MUFA are believed to enhance insulin signalling (Moon et al., 2010) whereas using omega-6 rich polyunsaturated oils may lead to an increase in arachidonic acid, which may down-regulate GLUT4 (Tebbey, McGowan, Stephens, Buttke, & Pekala, 1994). Both the Mediterranean diet and carbohydrate-restriction are also associated with a high degree of satiety (Paoli, Bosco, Camporesi, & Mangar, 2015; Schröder, 2007). Satiety may help to prevent overeating and allow longer periods of fasting.

Restricting carbohydrates have also been shown to confer additional health benefits compared to low-fat diets, especially with respect to weight, lipid profile, glycaemic control, and potentially kidney function (Ajala, English, & Pinkney, 2013; Bueno, de Melo, de Oliveira, & da Rocha Ataide, 2013; Hu et al., 2012; Juraschek et al., 2015). There are few large studies that compared the effects of carbohydrate restricted diets to the Mediterranean diet. However, restricting carbohydrates conferred greater weight loss, a larger decrease in triglycerides and hs-CRP, and larger increase to HDL after six months of dietary intervention (Shai et al., 2008). The Mediterranean diet favoured a decrease in fasting glucose in people with diabetes. The differences between the two diets had narrowed by 24 months but both showed improvements compared to a low-fat diet.

A key hyperinsulinaemia management strategy is to prevent hyperglycaemia and insulin secretion. This may explain the additional benefits to carbohydrate restriction. There are concerns regarding carbohydrate restriction, predominantly concerning high dietary fat. High fat consumption, especially saturated fat, is traditionally associated with adverse metabolic outcomes. However, studies conducted over two years have not found additional health risks (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005). Furthermore, high-fat dairy has been found to decrease the incidence of type 2 diabetes and the risk of death or hospitalisation due to coronary heart disease, compared to low-fat dairy (Holmberg, Thelin, & Stiernström, 2009; Kalergis, Leung Yinko, & Nedelcu, 2013). It is now believed that sub-types of saturated fats exist with differing health effects (Forouhi et al., 2014).

As with hyperglycaemia disorders, hyperinsulinaemia encompasses a range of severities. All three dietary strategies discussed above have the potential to improve the disorder. Logically, carbohydrate consumption in excess of what the body can tolerate, will invoke excessive insulin secretion. Therefore, restricting carbohydrates to a tolerated level should confer maximal health benefit, especially if the person consumes a whole-food diet based on Mediterranean principles. However, effective dietary management may be governed by adherence to the chosen regime (Boden, 2009; Pagoto & Appelhans, 2013).

Isolated beneficial nutrients / foods

Other compounds that have been shown to improve glycaemic control include magnesium, chromium, garlic, cinnamon, and green tea. Magnesium is believed to improve GLUT4 expression in rodent studies independently to insulin action (Solaimani et al., 2014). Chromium may improve insulin receptor sensitivity (Cefalu & Hu, 2004). There is some evidence to suggest many people are chromium deficient, especially if they eat highly refined foods, which, are not only unlikely to contain sufficient chromium, can also exacerbate its loss (Anderson, 1986). Emerging evidence suggests that magnesium and chromium may work synergistically to improve glycaemic control (Dou et al., 2015). Foods rich in these minerals are key components of the Mediterranean diet, especially nuts and whole grains. Green tea supplements, garlic and cinnamon (Hininger-Favier, Benaraba, Coves, Anderson, & Roussel, 2009; Padiya, Khatua, Bagul, Kuncha, & Banerjee, 2011; Solomon & Blannin, 2009) may also be beneficial for improving insulin sensitivity, but the mechanisms are not fully elucidated.

There are a number of traditional remedies for treating type 2 diabetes that may be beneficial for managing hyperinsulinaemia including (but not limited to) berberine (Lan et al., 2015; Yin, Ye, & Jia, 2012), fenugreek (Gaddam et al., 2015; Gupta, Gupta, & Lal, 2001), bilberries (Hoggard et al., 2013), black cumin (Hussein El-Tahir & Bakeet, 2006; Ramadan, 2007). While the mechanism of actions of these products are not fully elucidated, they are posited to include 5' adenosine monophosphate-activated protein kinase **(**AMPK), (berberine) similar to that of metformin (Zhou et al., 2001) or preventing carbohydrate absorption (bilberries, fenugreek). It is necessary to further assess the effect of these remedies on insulin release as both berberine and black cumin are posited to increase insulin release, although reports are mixed.

Medications

As previously stated, this review is predominantly concerned with hyperinsulinaemia in the presence of normal glucose tolerance. However, as people with impaired glycaemic control, are likely to be hyperinsulinaemic (Crofts, Schofield, et al., in submission), plausibly, strategies that improve glycaemic control without aggravating hyperinsulinaemia may optimise health. Other medications that affect hyperinsulinaemia may not be prescribed for metabolic disease, however, understanding this adverse effect is important.

There are two main medication strategies for managing hyperinsulinaemia: eliminating those that aggravate insulin resistance or contribute directly to hyperinsulinaemia; prescribing medications that improve insulin sensitivity. The latter should be considered second-line to lifestyle management. Medication management will be limited especially if hyperglycaemia, or other clinical conditions, need to be considered. For example, both antipsychotic medications, and longer courses of prednisone are known to aggravate insulin resistance and increase the risk of developing type 2 diabetes (Gonzalez-Gonzalez et al., 2013). However, stopping these medications in many patients may be inappropriate so alternative strategies need to be considered.

Medications that theoretically worsen hyperinsulinaemia

Medications may induce hyperinsulinaemia by: GLUT4 down-regulation; hyperglycaemia (via increased appetite, or affecting hormones such as adrenaline or cortisol); or directly increasing insulin secretion. These properties, especially GLUT4 down-regulation, may be difficult to discern from medication data sheets. If listed side-effects include weight gain or an increased risk of developing type 2 diabetes, then hyperinsulinaemia should be a reasonable suspicion. Medications known to down regulate GLUT4 include: clozapine (Heiser, Singh, Krieg, & Vedder, 2006); ritonavir (Vyas, Koster, Tzekov, & Hruz, 2010); statins (Sattar & Taskinen, 2012); and corticosteroids (Yuen, Chong, & Riddle, 2013).

Plasma insulin is increased by exogenous insulin, insulin secretagogues, or insulin mimetics, prescribed to manage hyperglycaemia. Although the insulin secretagogues such as sulphonylureas are less commonly used (Foster et al., 2013), little is known about the effects of these medications on hyperinsulinaemia. An unpublished case report suggests exogenous insulin used in type 2 diabetes can produce insulin spikes >

400 µU/mL for a number of hours following a 100 g glucose load as shown in Figure 10. The maximal insulin concentration remains unknown as the reference standard was only calibrated to a maximum of 400 µU/mL.

Despite this degree of serum insulin elevation, it can be noted that the patient did not attain a normal glycaemic profile. The combination of hyperglycaemia and hyperinsulinaemia increases the risk of a poor long-term prognosis for this patient. Further research is required to establish if this is an isolated situation or the standard response for many patients with type 2 diabetes.

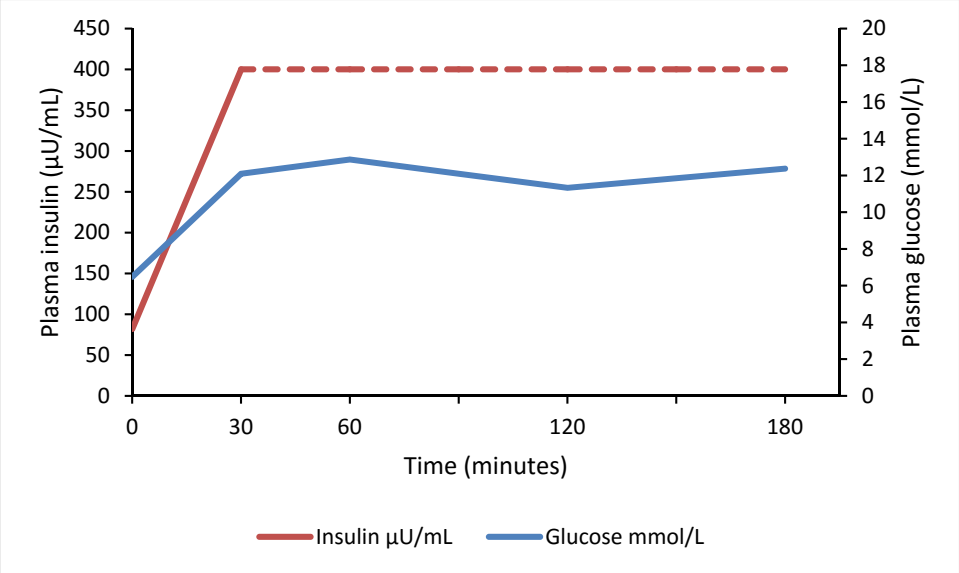

Figure 10: Insulin and glucose response in a patient with type 2 diabetes who received their normal morning exogenous insulin. The dotted line indicates the maximal range of the test.

Medications potentially beneficial for hyperinsulinaemia

Although the somatostatin analogue, octreotide, is used to treat isolated hyperinsulinaemia, (e.g. insulinoma) (Healy, Dawson, Murray, Zalcberg, & Jefford, 2007; Lustig, 2003), compensatory hyperinsulinaemia cannot be managed without concurrent glycaemic control. Hyperglycaemia is well recognised to have adverse pathologies, including diabetic ketoacidosis. But ketoacidosis can be triggered by low insulin levels independent of glycaemic status. Increasing levels of glucagon and cortisol may be triggered by cellular starvation, or hypoglycaemia. These hormones can induce gluconeogenesis and glycogenolysis leading to overproduction of the ketone bodies acetoacetic acid, β-hydroxybutyrate and acetone (Stojanovic & Ihle, 2011). Both acetoacetic acid and β-hydroxybutyrate are strong acids. Under normal circumstances

insulin levels help to regulate the production of these ketone bodies, but in its absence potentially fatal ketoacidosis may develop.

Thiazolidinedione-type insulin sensitisers, such as rosiglitazone, improve peripheral glucose uptake without increasing serum insulin levels (Kahn et al., 2000). However, all insulin sensitisers increase substrate uptake, which has implications for the formation of reactive oxidative species (ROS) and advanced glycation end-products (AGEs) and their adverse health effects (Ceriello & Motz, 2004; Nolan, Ruderman, Kahn, Pedersen, & Prentki, 2015). Furthermore, the use of thiazolidinediones is considered controversial because of their association with significant adverse effects such as heart failure, fracture risks, and increased risk of bladder cancer (Simon, 2013; Sinha & Ghosal, 2013).

Metformin is the most promising (albeit limited) medication to manage hyperinsulinaemia as it up-regulates GLUT4 (Zhai, Liu, Tian, Jiang, & Sun, 2012). However, unlike the thiazolidinediones, metformin also inhibits gluconeogenesis in the liver and/or delays glucose absorption from the gastrointestinal tract (Hundal et al., 2000). These latter actions may better reduce overall glucose load and therefore decrease endogenous insulin secretion. However, emerging research suggests metformin may not be beneficial for treating type 2 diabetes (Boussageon et al., 2012). Metformin may also cause excessive cellular nutrient uptake leading to increased ROS and AGEs (Nolan et al., 2015). Research does support the use of metformin for the treatment of polycystic ovarian syndrome, a condition associated with hyperinsulinaemia (Zhai et al., 2012). However, medication management of hyperinsulinaemia in the absence of another pathology cannot be supported by the current literature.

Novel mechanisms

Future targets for pharmacological management of hyperinsulinaemia may include insulin-degrading enzyme (IDE) and the forkhead transcription factor (FOXA-2). IDE mediates multiple hormones including insulin and glucagon. Rodent studies indicate impaired IDE, with resultant hyperinsulinaemia associated with poorer glycaemic control (Maianti et al., 2014). However, further research in this field may be able to selectively target glucagon. FOXA-2 has been shown to improve insulin sensitivity in a number of mouse models by controlling key genes in fatty acid oxidation and glycolysis (Puigserver & Rodgers, 2006).

Table 21: Summary of management strategies for managing hyperinsulinaemia

	Improved by	Worsened by	Indeterminate
Insulin receptor availability	Time Chromium MUFA	Hyperglycaemia Hyperinsulinaemia Highly refined foods	
GLUT4 up regulation	Magnesium Metformin Physical activity Time	Cortisol Excessive physical activity Arachidonic acid	
Hyperglycaemia	Carbohydrate-restricted diets Mediterranean diet Physical activity Black cumin	Excessive physical activity Excessive protein Excessive carbohydrate	High-carbohydrate, Low fat diets
Hyperinsulinaemia		Insulin Insulin secretagogues Insulin mimetics Very intense physical activity Excessive protein Excessive carbohydrate	Berberine Black cumin
AMPK activation	Berberine		
Reduced carbohydrate absorption	Carbohydrate-restricted diets Bilberries Fenugreek	Excessive dietary carbohydrate	
Mechanism unknown	Green tea Garlic Cinnamon		

Discussion

Hyperinsulinaemia is becoming recognised as an independent risk factor for chronic disease, yet there are few studies that address its management. This review evaluated hyperglycaemia management methods, including physical activity, diet, and medications while focusing on the mechanisms of hyperinsulinaemia as summarised in Table 21. First-line treatment of hyperinsulinaemia should encompass dietary and physical activity management. Physical activity should include a combination of aerobic and resistance activities, with an emphasis on HITT. Care is needed to avoid over-training, which may exacerbate insulin resistance. Further research is needed to understand how to obtain the optimal balance. With respect to diet, a carbohydrate-restricted Mediterranean diet theoretically confers greatest benefit but further research is needed, especially to determine to what degree carbohydrates need to be restricted in relation to the degree of hyperinsulinaemia. Although metformin may up-regulate GLUT4, pharmacological management is not currently justified due to the risks of cellular nutrition overload. Overall, strategies should aim to maximise participant adherence for greatest health benefits.

Chapter 8: Discussion

Metabolic syndrome is a significant risk factor for non-communicable disease and premature mortality. While the initial burden of metabolic syndrome was in developed countries, it is becoming more prevalent in developing countries. The "diseases of affluence" now perversely affect the poorest people. Public health measures for managing metabolic syndrome have had little impact on population health, as evidenced by the increasing prevalence of the condition.

Insulin resistance is recognised as a key component of metabolic syndrome although its diagnosis does not improve disease risk prediction calculations. Insulin resistance is generally associated with compensatory hyperinsulinaemia, often considered a symptom of insulin resistance. So far, there has been little consideration of hyperinsulinaemia as an independent risk factor for metabolic disease.

A broader investigation of this compensatory hyperinsulinaemia is warranted for several reasons. Understanding hyperinsulinaemia may present new direction for research into metabolic disease and offer new insights for treatment modalities. Diagnosing hyperinsulinaemia is problematic as there are no agreed upon reference standards. This thesis has outlined the first step towards a better understanding of hyperinsulinaemia and makes recommendations towards diagnosis and management.

Research summary and implications

Together with the conclusions from each separate chapter, this body of work contributes to the understanding of hyperinsulinaemia and metabolic disease in the following areas:

The health risks associated with hyperinsulinaemia extend beyond those traditionally associated with metabolic syndrome.

The links between metabolic syndrome and an increased risk of developing cardiovascular disease and type 2 diabetes are well-established as discussed in Chapter 2. This may be predominantly due to the effects that hyperinsulinaemia has on both vascular disease and pancreatic decay. That hyperinsulinaemia contributes to all forms of vascular disease has been recognised since the 1970s (Lauritzen, Larsen, Frost-Larsen, Deckert, & The Steno Study Group, 1983; Poulaki et al., 2002; Stout, 1990); even if the mechanisms are not yet fully elucidated. For macrovascular disease, it is recognised that insulin elevates triglyceride levels and depresses HDL cholesterol

(Stout, 1990). Emerging research associates a TG/HDL ratio < 1.0 being predictive of an Apo-A lipoprotein phenotype, which is associated with a decreased risk of coronary artery events (Hanak, Munoz, Teague, Stanley Jr, & Bittner, 2004). Insulin also stimulates lipid synthesis in arterial tissue, but not venous (Stout, 1990).

With microvascular disease, hyperinsulinaemia impairs fibrinolysis (Stegenga et al., 2006), meaning that microthrombi that may have formed will not be broken down. If these microthrombi lodge in peripheral capillaries, then peripheral vascular disease may result. This may be the cause of many diseases that are described as being "idiopathic" (no known cause). For example, primary hypertension comprises about 95% of all hypertensive cases and can be defined as hypertension occurring when there are no other secondary causes such as overt renal disease or pheochromocytoma present (Carretero & Oparil, 2000). It is plausible that sub-clinical peripheral or renal microvascular disease induced by hyperinsulinaemia is one of these causes; especially since first-line pharmacological treatment normally involves vasodilation (Gu, Burt, Dillon, & Yoon, 2012).

Other idiopathic conditions, including the middle ear disorders Meniere's disease and vertigo, probably have a multi-modal aetiology, which may include hyperinsulinaemia. Small studies have identified up to 68% of patients with Meniere's disease being hyperinsulinaemic compared to 13% of controls (Kirtane, Medikeri, & Rao, 1983). Hyperinsulinaemia is also associated with vertigo and migraines (Bhoi, Kalita, & Misra, 2012; Kazmierczak & Doroszewska, 2001).

Hyperinsulinaemia has the potential to aggravate cancer aetiology and proliferation from several different mechanisms. The somatic mutation theory of cancer aetiology suggests a series of mutations within the cell's DNA creates a cascade of events that leads to a cancerous cell (Sonnenschein & Soto, 2000). Amongst other mutagens, excessive production of reactive oxidative species (ROS) are believed to contribute to the initial mutations (Waris & Ahsan, 2006). Hyperinsulinaemia is associated with an increased production of ROS via cellular over-nutrition and increased Krebs cycle activity (Ceriello & Motz, 2004).

Hyperinsulinaemia is also associated with cancer cell proliferation through increased bioavailability of insulin growth factor-1 (IGF-1) (Sandhu, Dunger, & Giovannucci, 2002). Both insulin and IGF-1 receptors are found on cancer cells and *in vitro* studies

show that both these hormones can cause cancer cell proliferation (Pollak, 2008). This observation is also supported by population studies with type 2 diabetes conferring additional risk in the development of certain cancers, such as breast, or those associated with the alimentary system (liver, stomach, pancreas, or colon) (Giovannucci et al., 2010; Pollak, 2008). Hyperinsulinaemia is not associated with the development of some cancers, such as lung cancer, while the evidence remains inconclusive for others, including prostate and renal cancer (Giovannucci et al., 2010; Nandeesha, 2009; Tseng, 2012).

Hyperinsulinaemia, even in the absence of hyperglycaemia, is associated with cognitive decline in older people (Ahmed et al., 2014; Craft, 2009; Erol, 2008; Schernhammer, Hansen, Rugbjerg, Wermuth, & Ritz, 2011). Cognitive decline covers a spectrum of disorders including mild cognitive impairment, Alzheimer's disease, vascular dementia, dementia of Parkinson's disease, and frontal-temporal dementia. The prevalence of dementia is increasing, and more alarmingly, may affect more people under the age of 65 years than previously acknowledged (Ferri et al., 2005; Maslow, 2006). The potential burden of this disease is immense as those afflicted frequently require a very high degree of support especially if they are less than 65 years at time of diagnosis, tend to wander, or develop severe behavioural or psychological symptoms of dementia (BPSD), such as agitation or hallucinations (Freyne, Kidd, Coen, & Lawlor, 1999; Shaji, George, Prince, & Jacob, 2009). The mechanisms for these diseases are not fully elucidated, but are likely multimodal including vascular disorders, amyloid plaques, and glucose or other neurotransmitter regulation (Erol, 2008; Humpel, 2011; Irwin, Lee, & Trojanowski, 2013; Qiu & Folstein, 2006; Stegenga et al., 2006).

Insulin both crosses the blood brain barrier and a minor amount is synthesised within the brain. Instead of gluco-regulation, cerebral insulin appears to have more of a neuro-regulatory role (Erol, 2008). This has led to both hypo- and hyper-insulinaemia trialled as dementia treatments. Craft and colleagues (1996) showed that memory improvement was possible after infusing their patients with insulin and maintaining glucose at fasting baseline levels. By contrast Krikorian and colleagues (2012) showed memory improvement in people with mild cognitive impairment after following a ketogenic diet. Due to the nature of the memory tests used in the different studies, a direct comparison is not possible. Given energy dysregulation is considered a critical part of Alzheimer's disease (Erol, 2008), both extremes of glycolic flux may contribute to behavioural and psychological symptoms. In the hyperinsulinaemic state, more

glucose would be available as fuel, decreasing challenges associated with hypoglycaemia. Conversely, in the ketogenic state, there are lower levels of neurotoxic free radicals and higher levels of glutamate, a marker of neuron stability (Erol, 2008).

The literature reviewed in Chapter 7 suggests that carbohydrate-restriction may provide an adjunct treatment for conditions associated with hyperinsulinaemia. The degree of carbohydrate restriction may vary by condition. For example, migraines may be controllable with a moderate degree of carbohydrate-restriction, but cancer is believed to need nutritional ketosis for maximal benefit (Fine et al., 2012). As the symptoms of dementia have shown improvement with both hyperinsulinaemia and hypoinsulinaemia, determining the best diet is more problematic. Since both hyper- and hypo-glycaemia can cause more dementia symptoms, it also remains important for these patients to keep tight glycaemic control. The challenges associated with maintaining tight glycaemic control in people with type 1 diabetes, suggest it may be more may be more practical to offer a ketogenic diet to limit glycolic flux. While it is unknown if the ketogenic transition stage would accelerate disease progression, dementia is a terminal condition, often with poor quality of life for both the patient and the caregivers. If a ketogenic diet can offer better quality of life, then it should be offered as an adjunct treatment option. It is though acknowledged that it may be difficult to modify the diet in those patients with moderate dementia, and/or BPSD.

The above sections on cancer and dementia focussed on diet as the main strategy for lowering insulin. Physical activity should though be encouraged wherever possible, but may not be a practical strategy in those with compromised physical health.

The proportion of people with normal insulin tolerance and hyperinsulinaemia is likely wider than anticipated and potentially independent of obesity.

Obesity is a key factor in many metabolic diseases. However, these diseases still occur in non-obese people. Chapter 3 demonstrated that obesity is not associated with hyperinsulinaemia. This implies that while hyperinsulinaemia can be a key component of obesity, people with normal insulin levels can become obese, while people with hyperinsulinaemia may have a normal weight. The standard BMI limitations apply to this observation, especially those with normal insulin levels, as the data does not account for muscle mass, ethnicity, or waist circumference.

The traditional theory of obesity proposes that in many cases, obesity precedes the other metabolic changes (Figure 11). This theory implies that the obese person has volitional control over their weight; if they simply ate less and moved more, then they would better manage their weight.

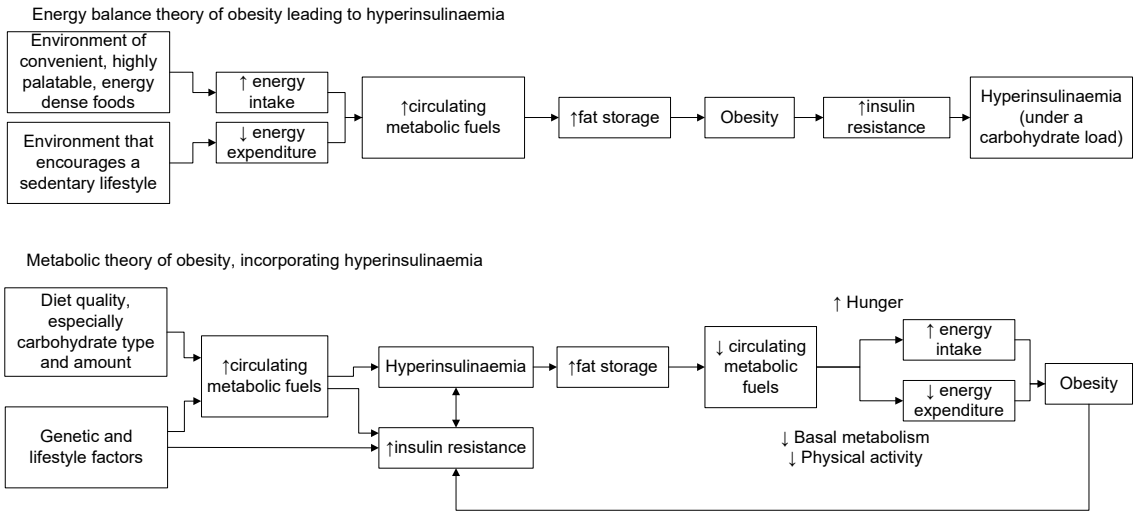

Figure 11: Traditional and metabolic theories of obesity adapted from Ludwig and Friedman (2014)

However, evidence is mounting that metabolic changes occur before weight gain. The metabolic theory of obesity suggests that hormonal imbalances occur before weight increases Figure 11. It is not clear which hormones are affected. While there is a focus on peripherally acting hormones such as leptin and insulin, there is an increasing body of knowledge in other hormones such as neurotransmitters, especially serotonin (5HT) and histamine, which are also known to affect appetite regulation and obesity. This phenomenon is well-demonstrated by patients commencing different antipsychotic medications that are known to cause weight increases. Plasma leptin increases were noted the week before a meaningful weight change for patients commenced on clozapine and the same week as those commencing olanzapine (Kraus et al., 1999).

This means that as a society, we need to think both beyond obesity as the first sign of disease and the use of BMI as the main measure of obesity. The limitations to using BMI are well-recognised as heavily muscled people, such as weightlifters or professional rugby players, usually have BMIs in the "overweight" if not "obese" categories while being metabolically sound (Thomas, Frost, Taylor-Robinson, & Bell, 2012). By contrast, people with normal BMI, but with significant central adiposity are recognised to have a higher mortality resulting from cardiac disease than those with a higher BMI, but a lower waist-to-hip ratio (Coutinho et al., 2013). There is also the

concept of the "TOFI", or "thin on the outside, fat on the inside". These people may not manifest the central adiposity aspects of metabolic syndrome, but have fatty liver disease, and lipid and insulin disturbances (Thomas et al., 2012). Unfortunately, in this latter group, medical imaging may be the only way to measure their internal adiposity.

People with impaired glucose tolerance or type 2 diabetes can be considered to be hyperinsulinaemic by default.

Chapter 3 showed that of the 1666 people with diabetes, 90% had a hyperinsulinaemic response (n = 1497) with the remaining 169 people having a "pseudo-normal" (Kraft I pattern) (n = 32) or hypoinsulinaemic response (n = 137). While the Kraft database does not distinguish between type 1 or type 2 diabetes, it is logical to assume people with type 1 diabetes will not have a hyperinsulinaemic response. Hyperinsulinaemia featured in 96% (n = 1697) of those with impaired glucose tolerance (n = 1762). This study clearly shows that the majority of people with type 2 diabetes or impaired glucose tolerance are hyperinsulinaemic and this may, in part, explain why these people are at increased risk of other metabolic diseases such as cardiovascular, or other vascular disease, cancer, or dementia.

Although hyperinsulinaemia was recognised to be associated with type 2 diabetes and impaired glucose tolerance in the 1960s (Berson & Yalow, 1961), by the mid-1970s, "maturity onset diabetes" was generally agreed to be a disorder of hypoinsulinaemia (Baird, 1973). The rationale for this decision is not clear. Some of the early studies that showed that "severe diabetes" was associated with hypoinsulinaemia, did not clearly distinguish between type 1 (juvenile) or type 2 (maturity onset) diabetes with respect to patient recruitment (Chiles, Tzagournis, & Catalano, 1970; Genuth, 1973). Also, despite showing that people with diabetes had an elevated insulin response to a glucose load, Perley and Kipnis concluded this should be a "hypoinsulinaemic response" due to insulin antagonism associated with obesity (1966).

Given the increased risk of hyperinsulinaemia for developing further metabolic disease, the implications of this for diabetes therapy are uncertain. Uncontrolled hyperglycaemia is highly damaging to all body systems. While tight glycaemic control is generally recommended for managing many diabetes complications, intensive insulin therapy and good glycaemic control are recognised as risk factors for the development of diabetic retinopathy (Lauritzen et al., 1983). Tight glycaemic control is also recognised as a risk factor for hypoglycaemia, which can have devastating effects on

brain function (Yaffe et al., 2013). Conversely, intra-cellular glucose and insulin deficiency, may lead to uncontrolled production of ketone bodies and resultant acidosis (Stojanovic & Ihle, 2011). As shown in Chapter 3, many people with impaired glucose tolerance or type 2 diabetes are hyperinsulinaemic. This means that clinical decisions in advanced metabolic disease with a number of co-morbidities may be a series of compromises between quantity and quality of life.

Hyperinsulinaemia should be redefined as an elevated post-prandial level.

Hyperinsulinaemia has not been well-defined throughout history. Many studies or clinical services use fasting insulin levels to define hyperinsulinaemic status with levels ranging from 6-30 μU/mL (Dankner et al., 2009; Kraft, 1975; Labtests, 2012; Lan-Pidhainy & Wolever, 2011; Odeleye et al., 1997; Waikato District Health Board, 2015).

Fasting insulin levels have also been used to identify those with either impaired fasting glucose, or impaired glucose tolerance, collectively termed 'prediabetes' (Johnson, Duick, Chui, & Aldasouqi, 2010). People in the highest quartile (mean insulin 25 μU/mL) were five times more likely to have prediabetes than those in the lowest quartile (mean insulin 5 μU/mL). An insulin level of 9 μU/mL had an 80% sensitivity and 42% sensitivity for diagnosing prediabetes. The benefits of this study are uncertain as fasting insulin was not compared to HbA1c; the current best-practice measure of impaired glycaemic control. There was also no discussion regarding the repeatability of fasting insulin, which this thesis has shown to be remarkably unreliable. Furthermore, this sensitivity and specificity result would miss a pre-diabetes diagnosis in 20% of people with an insulin level < 9 μU/mL or falsely diagnose 58% of people with an insulin level ≥ 9 μU/mL.

In Chapter 3, fasting insulin levels, for the majority of participants with normal glucose tolerance, did not correlate with post-prandial insulin levels (Table 9). Using the upper level of "normal" fasting insulin, approximately 80% of participants had an elevated insulin level (> 30 μU/mL) two hours following the 100 g glucose load. Even lower levels of fasting insulin do not predict resultant post-prandial hyperinsuliaemia as shown in Table 20, where 17% of those with a fasting insulin ≤ 5 μU/mL had a 2-hr insulin > 30 μU/mL. This suggests that there is a fundamental flaw in using fasting insulin levels to assess hyperinsulinaemia.

As discussed in Chapters 4 and 5, fasting insulin levels are ineffectual for diagnosing hyperinsulinaemia because insulin levels can fluctuate rapidly due to the pulsatile nature of insulin secretion. This means that, unlike many other hormones, it is very difficult to determine a basal level. But is a basal level necessary? Theoretically only high levels of insulin are associated with the damage as discussed in Chapter 2. Given an insulin bolus is released following a glycaemic load, understanding post-prandial insulin is more important than basal.

Parallels can be seen between diagnosing hyperinsulinaemia and hyperglycaemia. People with hyperglycaemia may have elevated fasting glucose and/or elevated post-prandial glucose levels. As many people only have the latter condition, prior to use of HbA1c, they could only be diagnosed using the two-hour oral glucose tolerance test. Using this parallel, until a new diagnostic test is developed, most cases of hyperinsulinaemia will only be diagnosed using a two to three-hour oral glucose tolerance test.

Focus should move from insulin resistance to hyperinsulinaemia

Research has traditionally focused on insulin resistance being key to metabolic disease, with the compensatory hyperinsulinaemia being relegated to a simple consequence of this condition. Chapter 2 describes the distinction between insulin resistance and hyperinsulinaemia (depicted in Appendix H). Insulin resistance can be defined as impaired glucose uptake while compensatory hyperinsulinaemia is the heightened insulin response required to restore euglycaemia. Yet, with the possible exceptions of fatty acid metabolism (Egan et al., 1996) or down-regulating hepatic gluconeogenesis (Barthel & Schmoll, 2003), only the direct effects of the hyperinsulinaemia can be mechanistically linked to subsequent disease.

Measures of insulin resistance such as HOMA do not improve disease risk calculations (Samaras et al., 2006; Schmiegelow et al., 2015). There is a plausible association between the degree of insulin resistance and the degree of hyperinsulinaemia required to compensate for the resistance (Table 17). Yet this may also depend on factors such as pancreatic health, and whether the person has retained first phase insulin secretion. However, the overarching question about insulin resistance is whether its relationship to metabolic disease has been superseded by hyperinsulinaemia.

Insulin resistance is a defence against overfeeding.

Chronic insulin resistance is associated with metabolic disease and a poorer long-term prognosis. However, metabolic versatility is required to maintain a normal physiology. Historically food intake and energy expenditure would vary in response to seasonal availability, pregnancy, illness, or trauma; possibly even age or societal status (Nolan et al., 2015). This meant that the body needed to be able to partition energy between tissues in response to available nutrients or energy expenditure. Insulin resistance may be a physiological adaptation to support energy partitioning. Short-term overfeeding causes transient insulin resistance in skeletal muscle, allowing excess nutrients to be stored in adipose tissues (Nolan et al., 2015). When this mechanism is overridden, such as by excessive insulin (exogenous or endogenous) cellular over-nutrition causes metabolic stress resulting in excessive production of ROS and advanced glycation end-products (AGE). These by-products weaken the cells in which they are formed. In the heart, this can lead to cardiomyopathy, with increased risks of cardiac failure. Therefore, acute insulin resistance could be a healthy mechanism. It is plausible that, in this state, hyperinsulinaemia does not occur, resulting in transitory hyperglycaemia. This would resolve by either glucose uptake into adipose tissues, and/or by glycogen depletion in skeletal and muscle cells.

The problem occurs when insulin resistance is prolonged. Sustained over-nourishment will result in drawn-out hyperglycaemia, which is associated with thrombus formation and nerve damage (Edwards, Vincent, Cheng, & Feldman, 2008; Stegenga et al., 2006). It is plausible that hyperinsulinaemia then occurs as it is associated with fewer risks compared to that of hyperglycaemia. Hyperinsulinaemia is a silent disease and generally remains undetected until hyperglycaemia and a glycaemic control disorder is diagnosed.

Hyperglycaemia management may override insulin resistance and cause cellular damage. Moderately severe glycaemic control disorders, such as impaired glucose tolerance, are initially treated with lifestyle management. Increasing physical activity is unlikely to aggravate hyperinsulinaemia. However, over-training and/or injuries can result in increased cortisol secretion, which down-regulates GLUT4 leading to increased insulin resistance (Björntorp & Rosmond, 1999). A low-fat diet, high in complex carbohydrates, may lower overall blood sugars, but may not sufficiently lower insulin levels.

Pharmacological treatment of hyperglycaemia is geared towards up-regulating GLUT4, either directly (metformin) or indirectly using insulin mimetics, secretagogues or exogenous insulin. This means that the medications overcome the cells' insulin resistance, causing over-nutrition, ROS and AGE production. In many cases, people use doses of insulin that are significantly higher than that found in a healthy person. While failure to treat hyperglycaemia has a 'very poor' prognosis, treating hyperglycaemia with supra-physiological insulin doses appears to have a 'poor' prognosis. This implies that to avoid many of these metabolic complications, hyperinsulinaemia should be diagnosed prior to detectable hyperglycaemia and that management should mimic normal physiology.

Limitations

These findings are circumscribed by several caveats. As discussed in Chapters 3 and 6, the Kraft database was collated over 20 years, commencing more than 40 years ago. It is not known whether these findings apply to the modern population. There are a number of gaps to the data that was collected.

Inclusion/Exclusion criteria

It has to be recognised that this was a convenience sample and may not be reflective of the population. There is an argument that due to the nature of the test, (i.e., identifying those with impaired glucose tolerance,) the database was skewed towards those whom the referring medical practitioners suspected were unwell. However, it must also be recognised that these same medical practitioners were Dr Kraft's colleagues and were very supportive of this research, to the extent that they, and their families, volunteered to be healthy controls (Kraft, 2014).

It is also unknown whether the patients had concomitant pathologies, (e.g. infections, neoplasia, or congenital diseases,) or were taking medications, which may have influenced the results. It is unlikely that anyone severely ill with a disease unrelated to a glucose tolerance disorder would have been referred for the test, and those with an acute, but mild, illness, such as a cold or flu, would have had their test deferred. Furthermore, many medications commonly used today were not common during the time of data collection. For example, aspirin was not used prophylactically for heart disease until the mid-late 1990s, while statins were not approved by the FDA until the late 1980s (Endo, 2010; Hennekens, Dyken, & Fuster, 1997). Nevertheless, this lack of

knowledge is a study limitation. By excluding people with a BMI < 18 kg/m^2, people who were severely ill with a concomitant pathology should be excluded, but this exclusion may skew the BMI data.

It was unknown which women were referred for an oral glucose tolerance test to determine glucose intolerances during pregnancy. Pregnancy would confound the results by two means: Pregnancy weight gain would confound the effects of BMI, while increased insulin requirements are a natural consequence of pregnancy (Kaaja & Rönnemaa, 2008) potentially confounding the insulin response pattern. Therefore, all women under the age of 45 years were excluded from this analysis. The age of 45 years was chosen as the probability of a natural pregnancy is low (Heffner, 2004) while assisted reproduction techniques were not common during this time period. Therefore, the extent to which these patterns apply in women of child-bearing age is currently unknown.

This study also excluded children and young adults under the age of 20 years. These participants received a glucose dose of 1.75 g per kg body weight up to a maximum of 75 g. This means the results cannot be directly compared to those receiving the 100 g dose. Therefore, as with women of child-bearing age, these results from this thesis cannot be generalised to anyone under the age of 20 years.

Insulin response patterns

This thesis carries forward Dr Kraft's theory that Kraft I pattern depicts a normal insulin response. While this theory is unproven, there is no reason to doubt it. This thesis defines a normal insulin response pattern as normal baseline insulin, with a moderate insulin elevation at 30-60 minutes, returning to normal levels by 120 minutes. This is the fundamental premise for normal glucose tolerance. It is recognised that future research will be needed to confirm this theory.

Few other researchers have looked at insulin response patterns in a similar way. As discussed in Chapter 3, Hayashi and colleagues investigated insulin response patterns formed following a 2-hour oral glucose tolerance test (2013). While this study clearly showed that an insulin peak at 120 minutes greatly increases the risk of developing type 2 diabetes, only the shape of the insulin response was investigated. It is yet to be determined whether the magnitude of the insulin response adds to the long-term outcome predictions. Furthermore, by truncating their study at two hours, the impact of

insulin peaks that occur at three hours or later could not be assessed. The long-term impact of very delayed insulin peaks (i.e. delayed beyond two hours) has yet to be assessed and this should be addressed in future research.

Assay precision

The Kraft database is based on medical technology that is over 40 years old. While it was the 'cutting edge' technology of its time, there are a number of limitations when compared to modern technology. This includes the assay precisions for both glucose and insulin.

Initially Dr Kraft measured glucose using the ferricyanide method, which had a documented CV of < 5%. During 1974, the glucose assay was changed to the Technicon method (also known as Johnson and Johnson, or Kodak). Kraft did not record either the exact date of change, nor the assay precision and this information is no longer available. Data reconstruction suggests an assay precision of < 3% (Purcell et al., 1979). The best date estimate of change to analysis method is between May and July 1974. This was estimated by assessing the raw data collected by Kraft and comparing to his publications (Kraft, 1974, 1975). Following the methods of Passey, Gillum, Fuller, Urry, and Giles (1977) as used by Dankner, et al., (2009), glucose levels were adjusted downwards by 10 mg/dL for glucose results for all results before May and again for all results prior to July 1974. After defining glucose tolerance disorders (World Health Organization, 1999) for each group and cross tabulating, approximately 30 participants tested between May and July 1974 were placed in a different glucose tolerance grouping with approximately one-third (n=11) changing between normal glucose tolerance and impaired glucose tolerance. For the purposes of this thesis, the date of assay change was deemed to be May 1974.

Insulin assays have their own inherent set of challenges as different assays can have disparate results for the same sample (Manley et al., 2007). Although all of Kraft's samples were analysed with the same method (the Phadebus Insulin Test) these results may not be directly comparable with other methods. Measured insulin concentrations are generally higher in serum compared to plasma. Although many of the available insulin assays do not cross react with proinsulin, it is currently unknown whether they can determine the difference between endogenous insulin and the various available or previously used human, porcine, or bovine recombinant insulins. The Phadebus assay used by Kraft did detect exogenous insulin as shown in Figure 5 (page 53).

This suggests that insulin levels, and/or insulin resistance measured calculated from these responses, determined by different studies may not be directly comparable between studies as due to the variety of techniques used. However, insulin response patterns can be compared between studies, with the caveat that the response magnitude needs to be assessed with caution.

Glucose dose

The oral glucose tolerance tests prescribes a fixed glucose load for every adult regardless of weight, height, or muscle mass. Following a glucose load, peripheral tissues preferentially adsorb the available glucose. This means that, assuming the same degree of insulin resistance, those with a greater muscle mass are more likely to require less insulin to be euglycaemic 120 minutes after test initiation. Certain tests, including the intravenous glucose tolerance test, use a glucose load calculated per kilo of body weight (0.3 g/kg) (Hovorka et al., 2002). Oral glucose tolerance tests conducted in children use a glucose load of 1.75 g glucose per kg of body weight up to a maximum of 75 g (World Health Organization, 1999). This means that anyone over 42.8 kg body weight gets the full 75 g dose.

Yet when lean body weights are calculated significant differences are evident, especially between sexes. For example, a man (1.8 m tall, 95 kg) has approximately 62 kg of lean body mass (Hume, 1966). By comparison, a woman (1.65 m tall, 65kg) only has approximately 45 kg lean body mass, yet is expected to dispose of the same amount of glucose in the same time period.

The Kraft database used a 100 g glucose load, which may both: a) be beyond physiological disposition; and b) cause gastrointestinal distress and delayed gastrointestinal empting, which may confound results. Future research should use a glucose load that is proportional to lean body weight in order to standardise glucose load.

Avenues for future research

This thesis has opened up many different directions for future research. Currently research in hyperinsulinaemia has been hampered by lack of a reference standard. For larger scale epidemiology studies, hyperinsulinaemia could be defined using the 2-hr insulin levels, whereas full Kraft patterning may aid understanding in disease progression.

Association between 2-hr insulin and long-term disease risk

Research should now focus on establishing the link between 2-hr insulin levels and risk of long-term disease. Hayashi patterns clearly show the link between having an insulin peak at 2-hr and an increased risk of developing type 2 diabetes, but this study did not take the magnitude of the peak into account (Hayashi et al., 2013). Focusing on the association between the magnitude of the 2-hr insulin peak and future risk of disease would determine these links. Reanalysing existing data would hasten results and limit costs or other resources, making it a more economical option.

Association between glucose response and hyperinsulinaemia

This thesis has focussed on the insulin response patterns produced after the consumption of a glucose load. These insulin responses can be described as dynamic in more than one context. This thesis has described insulin response patterns as dynamic as it has assessed the changes to the insulin response that follow the glucose load. However as can be seen from Figure 12 (page 128) the insulin response does not necessarily correspond to the glucose response pattern. For example, participant K2 had four different glucose response patterns but three different insulin responses.

Insulin is affected by a number of other hormones and factors including (but not limited to) cortisol, adrenalin, glucagon and the dynamic glucose response. This makes insulin a dynamic system by itself. What remains unknown is the extent to which the plasma glucose response to the glucose load subsequently influences the plasma insulin response.

It is plausible that further analysis of the glucose response patterns may be able to predict the risk of hyperinsulinaemia. As noted in Figure 4, people with a Kraft I or IIA pattern also tended to have a glucose peak at 30 minutes. The majority of research investigating disease risk prediction from a glycaemic response has focussed on either fasting glucose, or a two-hour response either post-prandial or following a glucose load.

Emerging research suggests that the shape of the glucose curve may predict insulin sensitivity with a biphasic, or even more complex curve shape being more likely to be associated with a higher degree of insulin sensitivity (Kim, Coletta, Mandarino, & Shaibi, 2012; Tura et al., 2011). However, using glucose response patterns to predict disease risk have similar limitations to using Kraft patterns; namely that of needing an frequently sampled oral glucose tolerance test of at least two hours in duration.

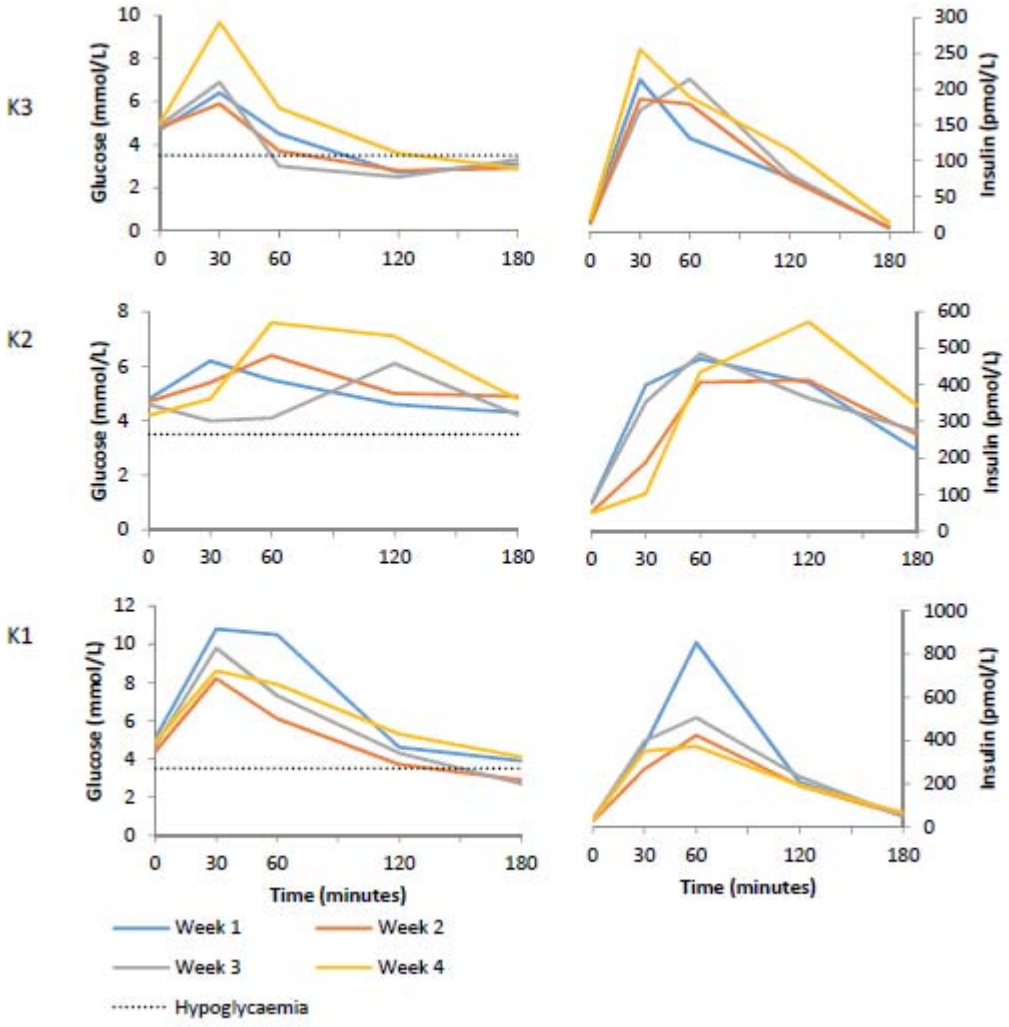

Figure 12: Glucose and insulin response patterns following four 100g oral glucose tolerance tests

Hypoglycaemia

Hypoglycaemic episodes (blood glucose levels < 3.5 mmol/L) can be noted in the study participants from Chapter 6 (Figure 12) Symptoms of hypoglycaemia include: hunger; shakiness; irritability; anxiety; confusion; sweating; or dizziness. All participants in this study were carefully monitored for signs of hypoglycaemia; but no signs were noted. The lack of hypoglycaemic signs, especially when blood glucose levels were < 3.0 mmol/L is interesting.

Hypoglycaemia is a major risk associated with the treatment of both type 1 and type 2 diabetes as it increases the risks of both mortality and morbidity. These risks may be due to clinical conditions such as increased cardiovascular risk, or neuronal death (Barnett et al., 2010; Schutz, 2011), or may result from injuries sustained from the

confusion or dizziness that can occur, including road traffic accidents and falls. Severe hypoglycaemia is associated with coma and death. Understanding why some people can be clinically hypoglycaemic, yet have no signs or symptoms of the condition may be important in reducing the burden of the condition.

It is believed that the signs of hypoglycaemia develop when the brain runs out of fuel. Ketone bodies, produced in the liver as a result of fatty acid oxidation, are an alternative source of fuel for the brain (Schutz, 2011). It is plausible that the study participants who had lowered levels of blood glucose, but no signs or symptoms of hypoglycaemia were oxidising fats and producing ketone bodies. Plasma concentrations of ketone bodies were not measured in the study participants. However, future research should re-visit the utilisation of ketone bodies for the prevention of hypoglycaemia (W. Johnson & Weiner, 1978).

Other potential diagnostic markers

Research should also address the possibility of other bio-marker/s, independent of a glucose load, which can also accurately predict hyperinsulinaemia. Several possibilities include using uric acid, liver enzymes, sex-hormone binding globulin, or upper-body fat distribution (Casassus et al., 1992; R. J. Johnson et al., 2009; Wallace, McKinley, Bell, & Hunter, 2013; Wannamethee et al., 2005). Currently, with the exception of HbA1c, these bio-markers are associated with the development of insulin resistance, but it is not yet confirmed whether these bio-markers are causal or the result of insulin resistance.

HbA1c is posited as another marker for hyperinsulinaemia. However, using this marker has number of fundamental challenges. The current understanding of hyperinsulinaemia is that insulin levels rise in order to maintain glucose homeostasis. This suggests that hyperinsulinaemia precedes elevations to HbA1c. Furthermore, HbA1c is a marker of average blood glucose over the previous 12 weeks. This means that episodes of hypoglycaemia may counter periods of hyperglycaemia. This suggests that a normal HbA1c cannot exclude hyperinsulinaemia, but a lower than expected HbA1c may indicate hypoglycaemic episodes that warrant further investigation.

Trends to HbA1c may also be an important diagnostic marker. There is current debate as to whether the diagnostic cut-off for determining glucose homeostasis disorders should be 37 or 40mmol/mol (Florkowski, 2013). Investigating trends to metabolic markers such as HbA1c, may be more important than using fixed-point cut-offs as a

trend may indicate an underlying clinical change that should be investigated. Further research is needed to confirm these theories.

Insulin area-under-the-curve and health risks.

As can be seen from Figure 13Figure 13: Plot of mean $AUC_{insulin}$ by mean BMI by Kraft patterns I-IV for participants with normal glucose tolerance a positive association can be noted between mean $AUC_{insulin}$ and mean BMI when assessed by the Kraft patterns in people with normal glucose tolerance. This association is less noticeable when the raw data is assessed (Figure 14), but the trend is still evident. As changes to $AUC_{insulin}$ are evident when BMI < 25kg/m^2, these results support the metabolic theory of obesity (Ludwig & Friedman, 2014) which suggests that hormonal changes, including hyperinsulinaemia, initiate the positive feedback cycle of obesity and further metabolic changes.

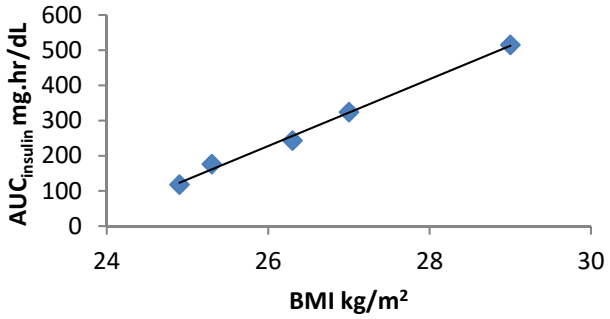

Figure 13: Plot of mean $AUC_{insulin}$ by mean BMI by Kraft patterns I-IV for participants with normal glucose tolerance

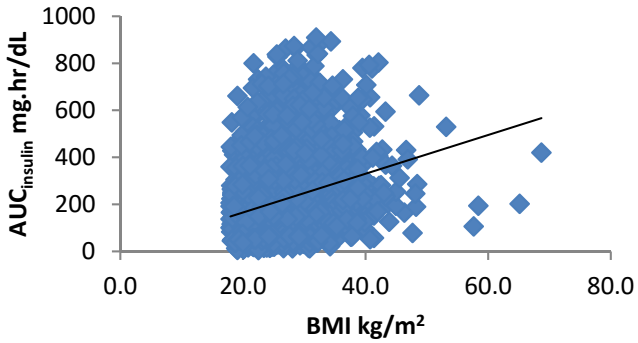

Figure 14: Plot of $AUC_{insulin}$ by BMI for Kraft patterns I-IV for participants with normal glucose tolerance

While obesity is recognised as being an independent risk factor for metabolic diseases (Hubert, Feinleib, McNamara, & Castelli, 1983; Manson et al., 1995), it is also recognised that not every obese person will develop metabolic disease (Chan, Rimm, Colditz, Stampfer, & Willett, 1994). This lack of predictability may be related to the insulin response. As evident in Figure 15, people may have the same $AUC_{insulin}$ following an oral glucose tolerance test with insulin assays, but their individual BMI and the shape of each curve may be very different.

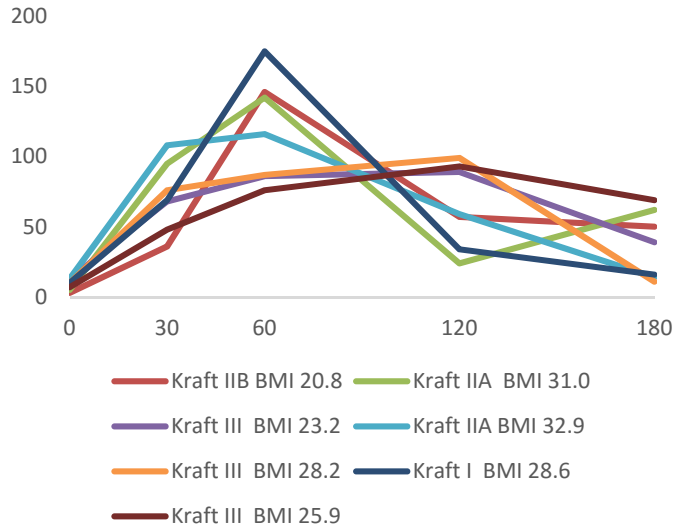

Figure 15: Seven individual Kraft pattern assessments in people with normal glucose tolerance with the same $AUC_{insulin}$

Hayashi et.al. (2013) demonstrated that the shape of the insulin response pattern is a significant factor in predicting the risk of type 2 diabetes (Figure 16). In their study, participants whose insulin concentrations peaked at 120 minutes following a 75g OGTT, had a significantly increased risk of developing type 2 diabetes (>38%) over 5-10 years compared with those with an insulin peak at 30 or 60 minutes (<16%). As the association between $AUC_{insulin}$ and disease risk prediction was not assessed, further research is required.

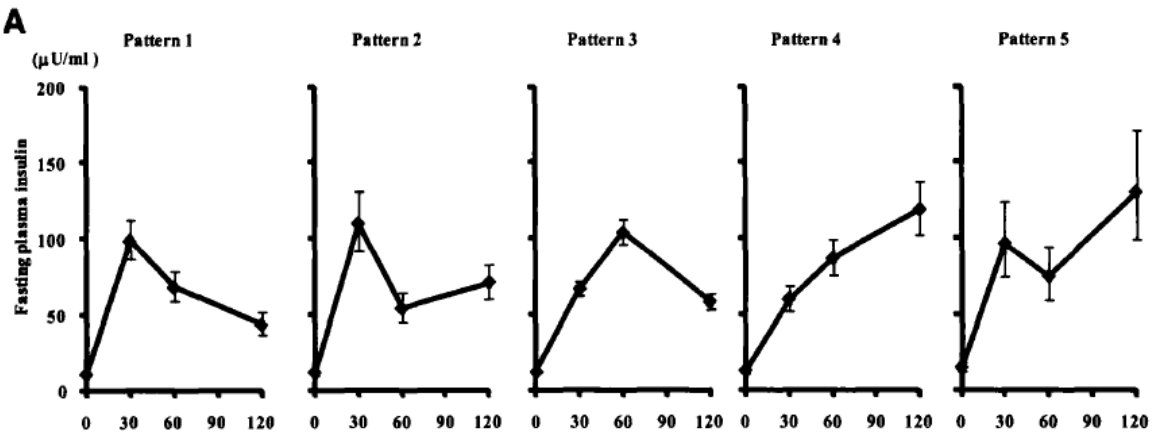

Figure 16: Hayashi insulin response patterns (reproduced from Hayashi et al. (2013))

Hyperinsulinaemia and non-traditional pathologies

If other similar data are available with different health end-points, such as cancer risk or cardiovascular disease, then the predictability of 2-hr insulin and these disease states should also be explored. Other pathologies, not just those associated with high, and/or early mortality should be explored. Middle ear conditions such as Meniere's disease and tinnitus are notoriously difficult to treat successfully yet have a significant impact on the patient's quality of life and may be associated with hyperinsulinaemia (Kraft, 1998). Understanding if these diseases are associated with hyperinsulinaemia may open up other treatment or research paradigms that may offer these people a better quality of life.

The benefits of managing hyperinsulinaemia on psychiatric and neurological conditions should also be explored. People with psychiatric conditions who are managed with anti-psychotic medications, such as chlorpromazine, haloperidol or clozapine, are more likely to have metabolic syndrome, compared to the general population (J. Meyer, 2004). This may be medication related for several reasons. These patients do not increase their risk of developing metabolic disease until after commencing medication (Mitchell, Vancampfort, De Herdt, Yu, & De Hert, 2013). Furthermore, these antipsychotics are described as increasing insulin resistance and are well-known as being a trigger for developing a glucose intolerance disorder (Taylor et al., 2007). Central adiposity and weight gain are significant contributors to these patients discontinuing their medication. Traditional diet and physical activity strategies are generally unsuccessful in managing the weight-gain in these patients. Worsening metabolic health can lead to conflicts in treatment between psychiatric management and physical health.

It is also plausible that hyperinsulinaemia management has positive implications for psychiatric conditions. Hyperinsulinaemia is implicated in many forms of dementia, especially from a vascular perspective but is also involved with the development of the plaques found in Alzheimer's disease (Craft, 2009; Monzo et al., 2013; Qiu & Folstein, 2006). Given the increasing prevalence of dementia, research that considers management options as well as prevention strategies should be considered.

Gestational and paediatric hyperinsulinaemia

Hyperinsulinaemia should also be investigated in the excluded patient groups, namely children and young adults under the age of 20 years, women aged between 20 and 45 years, and pregnancy. Type 2 diabetes used to be described as "maturity-" or "adult-onset" diabetes, because it was generally found in those aged greater than 40 years (Wilson, Hadden, Merrett, Montgomery, & Weaver, 1980). This is no longer the case. Currently, children, even those aged fewer than ten years old, are being diagnosed with type 2 diabetes to the extent that the type 2 diabetes is becoming more common than type 1 diabetes in Japanese children (Ehtisham, Hattersley, Dunger, & Barrett, 2004). Retinopathy is rarely present in people with type 1 diabetes prior to their diagnosis (Donnelly et al., 2000), but may be present in 20% of those newly diagnosed with type 2 diabetes (Fong et al., 2004). This means that these children are more likely to be affected by vascular consequences of diabetes compared to children who are diagnosed with type 1 diabetes at the same age. Early onset of diabetes-related vascular complications presents a significant societal burden due to impact to healthcare, economic development and the individual's quality of life.

Future treatments

Further treatment options need exploration. As previously discussed in Chapter 7, lifestyle management, especially diet and physical activity offers the best theoretical treatment options for hyperinsulinaemia. Pharmacologically reducing plasma insulin without ensuring euglycaemia has a poor prognosis. However, lifestyle options should take a patient-centred approach. This thesis has clearly shown that different people are affected by hyperinsulinaemia to different degrees and therefore, a "one-size fits all" management strategy is unlikely to be effective. To manage hyperinsulinaemia, there needs to be a balance between carbohydrate and protein intake, glucose/glycogen expenditure and insulin receptor and GLUT4 regulation.

Optimal management will differ for different people. Those with a Kraft I pattern should be able to maintain their healthy insulin response by eating a whole food diet with adequate protein, avoiding foods with added sweeteners and being moderately physically active. People with increasing levels of hyperinsulinaemia will require additional energy expenditure, especially a combination of high-intensity interval, and resistance training, and/or a greater degree of dietary management, especially carbohydrate restriction as described in Chapter 7. Most people will require a combination of dietary management and physical activity as these appear to have synergistic effects for controlling insulin levels. What is currently unknown is the degree of change required for each person. Someone with a normal fasting level, but delayed insulin peak (e.g. Kraft III pattern) may need a different approach to someone with an elevated fasting level but 60-minute peak (i.e. pattern IIA, or IIB). Further research is required to address this understanding.

This means that many different dietary or physical activity strategies may be appropriate for different patients, but also for the practitioners advising them. Many current practitioners have grown up in the "low-fat, whole grain" era, and may be uncomfortable with low-carbohydrate, high fat recommendations; especially those that suggests that higher amounts of saturated fats are not unsafe (Siri-Tarino, Chiu, Bergeron, & Krauss, 2015). For these practitioners, using a Mediterranean diet strategy which lowers carbohydrates but emphases olive oil, nuts, vegetables and legumes may be more appropriate.

Lifestyle management of hyperinsulinaemia may extend into the gut microbiome. A healthy and varied gut microbiome is responsible for many aspects of metabolic health, including the manufacture of a number of vitamins including B-12, and K (LeBlanc et al., 2013). Poor gut microbiota is associated with obesity, but it is now hypothesised that the gut microbiome directly impacts insulin resistance as well (Caricilli & Saad, 2013). The mechanisms are not fully understood but may include altering the absorption of ingested carbohydrate (either by fermentation or by bacterial use in the gut), changing intestinal permeability or increasing inflammatory pathways.

Bacteria that produce short-chain fatty acids are especially important for gut health as this is believed to impact bowel inflammation. Seven days of ciprofloxacin severely depleted these bacteria resulting in a deficiency of short-chain fatty acid production in the gut for at least 12 months after exposure (Zaura et al., 2015). The effects of other

antibiotics ranged from no significant effect (amoxicillin) to restoration at one month (minocycline) or four months (clindamycin). The effects of repeated courses of these antibiotics are unknown, but there is an association between antibiotic use during infancy (0-2 years) and obesity in early childhood (2-5 years) (Bailey et al., 2014). Further research is needed to establish whether restoration of these bacteria should be recommended following antibiotic use, especially those with prolonged effects, such as ciprofloxacin.

The antibiotics used by Zaura et al. (2015) are representative of the common antibiotics used in New Zealand. It is perhaps fortunate to note that the antibiotics with less effect on bowel health are, or represent, the ones more commonly used in primary medical practice (amoxicillin, minocycline), while ciprofloxacin and clindamycin are reserved for more specialised indications. Yet in many developing countries, these antibiotics are widely available and may be purchased for use without a prescription, or any guidance for appropriate use. Traditionally this raises concerns for antibiotic resistance and the emergence of bacterial infections that do not respond to antibiotics. Now it must also be questioned if unrestrained use of these, or similar antibiotics, contribute to the development of metabolic syndrome.

Technology

Research into the long-term effects of hyperinsulinaemia and respective management strategies are currently hampered by the difficulty of acquiring the plasma sample and costs of analysis. Development of point-of-care devices and/or cheaper means of analysis would extend this research.

The material addressed in Chapter 7 demonstrates that we cannot maintain insulin levels by pancreatic control without considering concurrent glycaemic control. This limits current pharmaceutical options. However, novel pharmaceuticals are targeting hypothalamic regulation of appetite (Halford & Harrold, 2012). Centrally acting agents are normally limited by unacceptable side effects. Yet further understanding of the effects of the insulin as a neurotransmitter, may provide further treatment paradigms.

From research to practice

Hyperinsulinaemia is a spectrum

Hyperinsulinaemia describes a spectrum of disorders as shown by the range of Kraft insulin response patterns. In people with a normal insulin response, insulin secretion is believed to be biphasic. During hyperinsulinaemic-euglycamic clamp studies, after a glucose bolus, there is a rapid first-phase insulin release peaking at 2-4 minutes, falling to a nadir at 10-15 minutes, then gradually increasing and persisting for the duration of the stimulus (Gerich, 2002). The latter insulin increase represents second-phase. In people with impaired glucose tolerance or type 2 diabetes, first-phase response may be diminished or non-existent.

The presence or absence of first-phase response can be seen in Kraft patterns (Figure 4). Following an oral glucose tolerance test, people with a healthy first phase response have an insulin peak at 30-60 minutes and a relatively rapid decline (Gerich, 1997). This equates to a Kraft I pattern. A Kraft III pattern, with an insulin peak at 2 hours or later, represents a loss of first-phase.

Although lack of the first-phase response is believed to be one of the earliest signs of impaired pancreatic function (Gerich, 2002), this does not explain the Kraft II or Kraft IV patterns. Nor does it explain the Hayashi patterns as shown in Figure 17**Error! Reference source not found.**.

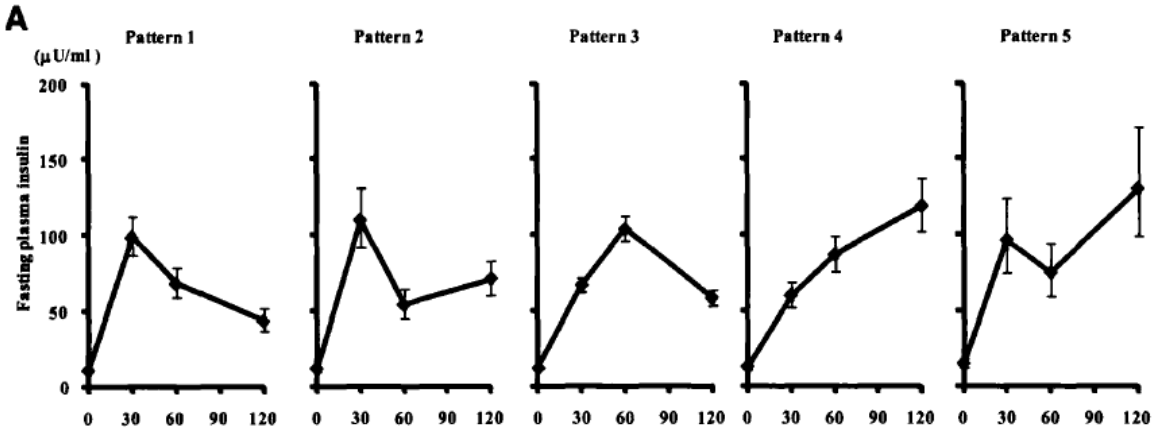

Figure 17: Hayashi insulin response patterns (reproduced from Hayashi et al. (2013))

Patterns 1, 2 and 5 have an insulin peak at 30 minutes suggesting first-phase insulin response, but the differences in the 120 minute levels indicate further differences in physiology. This is reflected in the 10-year cumulative incidence of diabetes of 3.2%,

9.8%, and 37.5% respectively. Pattern 4 has an insulin peak at 120 minutes, suggesting a loss of the first-phase response and had a 10-year cumulative incidence of diabetes of 47.8%. Of interest, pattern 3 has an insulin peak at 60 minutes, but has a 10-year incidence of diabetes of 15.4% - a figure that lies between those with a 30-minute or a 2-hour peak. This further suggests that there may be more than one pathological process contributing to the development of impaired glycaemic control as suggested in Chapters 2 and 7.

These differences in insulin response further suggest that individualised management strategies may be required as people with different degrees of hyperinsulinaemia will likely respond to different degrees of carbohydrate restriction, or even types of carbohydrates. Consumption of low GI carbohydrates in those lacking a first-phase insulin response may result in a prolonged insulin response as the body copes with a gradual glucose influx. This may be particularly marked in those who consume simple carbohydrates in combination with complex carbohydrates, such as porridge with fruit or honey. Further research will be needed to confirm.

Metabolic flexibility and carbohydrate restriction

Metabolic flexibility is defined as the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions (Kelley & Mandarino, 2000). People with insulin resistance become metabolically inflexible and are unable to change easily between fuel sources. The degree of metabolic inflexibility (as assessed by respiratory quotient) is associated with the severity of insulin resistance. This suggests that the ideal lifestyle should support metabolic flexibility.

Anecdotal evidence suggests that people embark on low-carbohydrate diets for a variety of reasons, including weight loss, or the prevention or treatment of impaired glycaemic control or hyperinsulinaemia. Before changing diets, these people are generally metabolically inflexible (and insulin resistant) as evidenced by a history of impaired glycaemic control. They hope that, by changing their diet, they can maintain normoglycaemia and prevent hyperinsulinaemia. While there is evidence to suggest that lifestyle change can restore metabolic flexibility, it is currently unknown whether restricting carbohydrates will restore their insulin sensitivity and/or pancreatic function. Plausibly, depending on the severity and duration of their disease, insulin sensitivity

will be restored, but restoration of pancreatic function will depend on the health of their β-cells.

Currently, the degree of carbohydrate restriction varies from mild (i.e. avoiding added sugars), to extreme carbohydrate restriction (i.e. < 20 g/day), with the latter aimed at maintaining a state of nutritional ketosis (plasma ketone levels 0.5-7.0 mmol/L). Many people opt for nutritional ketosis as the presence of adequate ketones in the presence of normoglycaemia suggests a Kraft V (hypoinsulinaemic) insulin response pattern. Further research is needed to confirm this theory. There is a significant amount of evidence to support the use of carbohydrate-restricted diets in people with confirmed metabolic disease (Chapter 7). However, there is no evidence to suggest restricting carbohydrates beyond minimising added sugars and refined carbohydrates will prevent the onset of metabolic disease.

It is generally assumed that there are no long-term ill-effects from chronic hypoinsulinaemia as the diseases associated with hyperinsulinaemia will be avoided. Short-term studies have not found any risks (Dashti et al., 2004). However, the risks associated with chronic hypoinsulinaemia in the presence of normal glucose tolerance remain unknown. Some people report high levels of satiation and as a result may reduce their caloric intake (Paoli et al., 2015). Plausibly this will induce insulin resistance via starvation pathways and render the person less able to manage a glucose load. Could this also be defined as a degree of metabolic inflexibility?

This then raises the question of whether there are health consequences for people with normal glucose tolerance in long-term nutritional ketosis. Evidence does support nutritional ketosis for known therapeutic purposes, such as an adjunct treatment for type 1 diabetes or epilepsy (Paoli, Runbini, Volek, & Grimaldi, 2013). However, on the basis of the current literature there is little evidence to support chronic nutritional ketosis in otherwise healthy people.

Most hormones need to be managed within a therapeutic window where levels either too high or low cause a disease state (e.g. hypo- or hyper-thyroidism). This suggests that insulin 'spikes' as demonstrated by a Kraft I pattern insulin response pattern may be essential for maintaining good health. Maintaining chronic hypoinsulinaemia will prevent these spikes. There are well-established concerns that maintaining therapeutic ketosis in children with epilepsy is associated with growth retardation (Yin et al., 2012).

For these children, the benefits of the diet outweigh the risks, however, the effects in adults are unknown. This means that, until further research confirms long-term safety, chronic nutritional ketosis should not be recommended without a therapeutic purpose.

Periodic nutritional ketosis, could be considered as an alternative to chronic nutritional ketosis. Many different populations around the world have intermittent fasting or periods of ketosis as part of their lifestyles including many religions and nomadic populations. While the periods of ketosis would vary, the key for these populations is the maintenance of metabolic flexibility.

Consuming carbohydrates when carbohydrate-intolerant.

Currently the majority of people are advised to follow the same diet to optimise health. Like many other health authorities, the New Zealand Ministry of Health advocates a moderate to high carbohydrate and low-fat diet (Ministry of Health, 2015). While these guidelines are aimed at the general population, and may maintain healthy metabolic regulation, they may not be optimal for the large percentage of the population who are metabolically dysregulated. Recently it was estimated that up to 14% of Americans had type 2 diabetes while a further 38% had either impaired fasting glucose and/or impaired glucose tolerance (Herman & Rothberg, 2015). Although these results may not yet apply to New Zealand, the overall global trend of an increasing prevalence of glycaemic control disorders is concerning. The question remains as to whether public health dietary strategies should be aimed at maintaining health, or should they be aimed at managing a moderately hyperinsulinaemic population?

While the latest recommendations have seen improvements in the advocacy of whole, unprocessed foods, the endorsement of the high carbohydrate, low fat message still exists. A low saturated fat diet is also still endorsed with recommendations to use low-fat dairy products and lean meats, and to use margarine and vegetable oils instead of butter / coconut oil or lard. Despite evidence that an imbalanced omega 3:6 ratio may be metabolically damaging (Simopoulos, 2008), there is no suggestion that monounsaturated fats should be preferred. Those trying to avoid gaining excess weight or to lose weight are recommended to “choose nutritious foods that are low in energy” (for example, with very little fat and no added sugar). The latest recommendations are still prescriptive regarding the minimum number of servings of different food groups each day including: six servings of grains; three of vegetables; two each of (low-fat) dairy and fruits; and little to no added sugar.

As carbohydrates are well-recognised to cause hyperglycaemia, diabetes has been described as "a disease of carbohydrate intolerance" (Feinman, 2016). Logically then, as with other food intolerance disorders, restricting carbohydrate should be first-line treatment. Improvements to HbA1c, fasting glucose and triglycerides occur after even moderate decreases in carbohydrate consumption (Kirk et al., 2008). Yet some groups argue that carbohydrate-restricted diets are too strict for those with diabetes. For example, while recognising that sugar has no nutritional value, Diabetes New Zealand believes small amounts of sugar are acceptable as part of a healthy meal plan (www.diabetes.org.nz). People are encouraged to have three to four pieces of fruit throughout the day, a teaspoon of sugar or honey is considered acceptable on unsweetened porridge, and high sugar foods such as ice-cream, cakes and biscuits are considered acceptable if limited to no more than twice a week.

These contradictions are difficult to understand. Given the greater number of medications available to manage hyperglycaemia, the immediate impact of high-sugar foods is lessened. Yet, the same logic does not apply to those with allergic reactions to food. We do not tell someone with a peanut allergy to “enjoy the peanuts, just remember to have your adrenaline injection and antihistamines handy.” Admittedly, someone having a sugary treat is unlikely to have an immediate life-threatening reaction, but the resultant damage is difficult to quantify. The questions remain as to whether people with diabetes should be counselled permissively with regard to carbohydrate treat foods given the resultant exposure to hyperglycaemia and/or hyperinsulinaemia with potential for adverse reactions?

Kraft tests versus post-prandial assessments

The Kraft test occurs in an artificial situation. After a 10-12 hour fast, the person consumes 100g of glucose in an aqueous solution, then does not consume anything else for a further 3-5 hours. This does not reflect day-to-day life and the highly standardised test conditions means that the Kraft test can only be considered a clinical tool. How we use the Kraft test in clinical practice is debatable. For example, a person commences carbohydrate restriction in order to manage their hyperinsulinaemia as diagnosed by a pattern IIB. They consume about 80g carbohydrate a day (predominantly from low GI non-starchy vegetable sources) and have increased physical activity. Metabolic improvements are monitored over the next six months with noted improvements to triglyceride levels, weight and girth. The question then remains as to whether their

insulin patterns should be re-measured? To repeat the standardised Kraft test, the person would have to consume an increased amount of carbohydrate for the two weeks prior to the test. Would this increase in carbohydrate consumption, including the 100g glucose required for the test itself, negate some of the benefits of the previous six months?

Instead, we should consider that the Kraft test be reserved for the initial assessment of hyperinsulinaemia status in people with normal glucose tolerance. As shown in Chapter 3, people with known impaired glucose homeostasis, such as impaired glucose tolerance, can be considered hyperinsulinaemia by default, therefore Kraft testing may not be suitable.

Ongoing monitoring should assess the effectiveness of hyperinsulinaemia management strategies in real-time and real-life. Ideally, we should assess whether insulin has returned to a baseline level two hours after each meals. This thesis suggests that the baseline level should be < 30 μU/mL. This means that the person has real time feedback as to whether their current lifestyle combination of physical activity and diet is effective at managing their hyperinsulinaemia. Currently an insulin level can be monitored two hours following breakfast, or lunch by using a community pathology laboratory. But the results may not be available for 24-48 hours. Therefore, for effective real-time monitoring of insulin levels we need a point-of-care device for the patient to self-monitor their insulin levels.

This strategy is essentially the same as that for blood glucose monitoring for people with diabetes. After an initial diagnostic test performed under standardised conditions, the person normally receives advice to manage their condition, and a point-of-care device for real-time monitoring. This means the person receives immediate feedback as to what strategies do, or do not, effectively control their blood glucose levels.

Informed choice

The nature of academia is that of debate and discussion of different theories, especially when new evidence appears to contradict an existing theory, or where debate has continued for years. In the past, these debates and discourses generally occurred either through academic journals or at conferences. Until relatively recently, laypeople were excluded from these debates, unless they were highly motivated and had access to the

journals. This access changed with the “Information Revolution”. Now, almost everyone has access to the Internet and “google” is an accepted verb. Many journals are "open access" so subscriptions are not required and anyone can read research from wherever they have access to the internet. This means that patients have access to cutting-edge information; they access it themselves or are exposed to it by others in online forums and blogs where laypeople discuss research and share personal experiences. In this modern technological era, it is virtually impossible to hide information from the public eye. Patients are sourcing the latest information about the different treatment options for their conditions and may have accessed more research regarding their condition than their health practitioner. Some practitioners will find this disconcerting and challenging especially those who are time-poor and rely on their professional bodies to keep them updated. General practice practitioners cover very broad clinical areas and would find it even more difficult to stay up-to-date with the latest research for all of their patients’ conditions. Yet patients generally lack the skills to fully interpret and understand the latest research; and more importantly to understand how this research applies to them specifically. This means that the average patient needs support and advice to be able to exert their right to be fully informed. This may represent a significant change to health care practices in the future.

The New Zealand Health and Disability Code of Rights (Health and Disability Commissioner, 2004) advises that the patient has the right to make an informed choice about their treatment (Right 6). These rights include an explanation of the options available (Right 6.1b) and the results of research (Right 6.2d) provided in a manner that respects their dignity and independence (Right 3). They may also refuse treatment (Right 7.7), but maintain the right to be treated with respect (Right 1.1), free from discrimination, coercion or harassment (Right 2).

This means that a patient should be able to research and/or have recommended to them alternative treatments to their condition and have a frank, honest, and respectful conversation with their health care provider about different but viable options. This is of particular importance with lifestyle measures such as diet and physical activity since adherence is vital for success. Yet these conversations do not seem to occur. Anecdotal evidence and observations from health advocacy websites such as Diabetes New Zealand and Dietitians NZ suggest that the high-carbohydrate, low-fat diet is the default recommendation for optimal health. These organisations could suggest in their online communication that other approaches, including the Mediterranean or

carbohydrate-restricted diets, are effective and issue caution that implementation need to be alongside a suitable health practitioner. However, this is not the case, and in fact at times these organisations are dismissive of the low-carbohydrate, high-fat approach. The specific reasons for maintaining the high-carbohydrate, low-fat preference are unclear but the observation does raise the question of whether informed choice for diet actually occurs in practice.

Part of the answer may lie in the New Zealand Dietitians Code of Ethics which appears to provide contradictory information (http://dietitians.org.nz/fileadmin/assets/Member_Admin/Code_of_Ethics_and_Conduct_October_2014_web.pdf).

The first part of the code specifically states that dietitians must base their professional decisions on the following principles:

1. Autonomy - The right of consumers to make their own choices, after receiving objective evidence-based information, must be respected;
2. Beneficence – You must act in the best interest of the patient
3. Non Maleficence - You must not cause harm.
4. Justice - You must act fairly and provide services in an objective, non-discriminatory and unbiased manner.

Yet in the latter part of the code, dietitians may not advertise claims for the health benefits of products or services (Principle 5b), or advertise claims that one product, brand or service is better than another (Principle 5d) unless these are supported by scientific evidence that has been published in a reputable source. The example given of the reputable source is the Ministry of Health Food and Nutrition Guidelines. Furthermore, the advertising of products brands, foods or services can only occur if all of the following occurs:

1. Protect and promote the health and wellbeing of the New Zealand public.
2. Have been shown by scientific evidence that has been published in a reputable source to have health benefits.
3. If they are foods, then they are everyday foods in amounts that support the Ministry of Health's Food and Nutrition Guidelines
4. Do not undermine the Ministry of Health's Food and Nutrition Guidelines.
5. Comply with the Fair Trading Act.

The footnotes in the code state that these definitions exclude "material issued to patients or clients during consultations where such material is designed to provide the patient or client with clinical or technical information about dietetics or health conditions and

where the patient is afforded sufficient opportunity to discuss and ask questions about the material. Also, this definition is not intended to apply to material issued by a person or organisation for the purpose of public health information or as part of a public health programme."

This suggests that in private consultations dietitians can recommend carbohydrate restriction as an optimal diet for people with hyperinsulinaemia, but cannot promote it in a public forum as this may be seen as advertising. Having a different private opinion compared to a public option is often described as hypocritical. This may be actively stifling diet debate for dietitians, our key health professional providers of dietary advice. Given many people cannot afford to see a dietitian privately, there is a high reliance on publically available information. Patients researching dietary options will not be receiving objective evidenced based material if only one dietary recommendation can be advocated in public by our dietary experts. The question remains whether patients' rights are being breached if a well-researched option, that consistently outperforms mainstream guidelines, is not publically discussed.

This raises the concern of patients losing confidence in dietitians. After the patient has researched the options and decided that restricting carbohydrates may be beneficial, but wants professional assistance, they will not be able to find a dietitian advertising this service. What they will find is a variety of nutritional advisors who are advocating the patient's chosen dietary strategy. These nutritional advisors are generally not registered health professionals, and may or may not have sufficient knowledge and/or experience to optimally deal with certain metabolic diseases. Alternatively, anecdotal evidence suggests that many patients turn to peer-support groups that are often found on social media websites.

If our dietitians are not recommending alternative dietary strategies, then other healthcare providers may also not feel comfortable with these discussions with patients. Conversations with other New Zealand healthcare service providers suggests that, in many cases, they can see the logic in restricting carbohydrates, but there are a number of barriers towards implementation. They do not feel sufficiently informed about the topic and there appears to be little guidance from professional bodies. Most practitioners do not have the necessary time and academic resources to be able to fully research the topic. Furthermore, there are challenges to implementation. For example, a motivated patient may decide that carbohydrate restriction may be a viable adjunct

treatment for their type 2 diabetes. Due to their current medication regime and insufficient nutritional knowledge, they will need both a prescriber, to manage the likely medication changes, and dietetic support. Finding a combination of health care providers who are prepared to work as a team towards this patient-centred goal may be difficult.

Practitioners may also feel a high level of cognitive dissonance by being asked to do almost the exact opposite of their usual practice. But ethically, what is the solution? Is it fair to let the patient flounder by themselves, or would it be better for the practitioner to say that although they disagree with the diet choice, they will agree to monitor the results and review the patient's choices if there is evidence of adverse effects?? Currently, there is no evidence that over two years, a low-carbohydrate, high-fat diet has adverse metabolic effects (Gardner et al., 2007). Given only about 20% of patients succeed in losing 10% of body weight over 12 months using lifestyle interventions (Wing & Phelan, 2005), this suggests the majority of patients will need additional support. Therefore, most patients will need at least an annual health and lifestyle review where adherence can be supported and reinforced. At the same time, any metabolic changes can be noted and treatment adjusted as appropriate.

Public domain

There is an argument that debates about what constitutes a healthy diet should be kept out of the public domain because it confuses the public. Yet, when the differing options are considered, there is a wide range of overlap:

- The majority of the diet should be plant-based whole-foods, with moderate protein.
- Products high in refined carbohydrates and added sugars should be avoided.
- Healthy fats are necessary.

Where the dissent occurs is in the details, especially the proportions of macronutrients or the inclusion/exclusion of certain foods or nutrients, especially grains, seed oils, and saturated fats. Previous research has shown that focusing on a single public-health message for weight loss resulted in very similar clinical outcomes to a multi-component message at 12 months (Ma et al., 2015). This means that if the main public health message focuses on maximising whole-foods and minimises processed foods, the public will receive a more cohesive message. Significant health improvements are proposed if

people simply consume less refined ingredients and processed foods, which are often energy-rich, but nutrient-poor.

Conclusion

The prevention and management of hyperinsulinaemia represents a number of challenges for health agencies. The diagnosis and treatment options presented in this thesis depart from current practice. Diagnosing hyperinsulinaemia means a return to using a two-hour oral glucose tolerance test, a test less frequently used compared to HbA1c, which demands fewer resources. Carbohydrate restriction is a controversial topic as it often means increasing dietary fat, of which many agencies do not approve. However, the biggest challenge is for public health to accept that hyperinsulinaemia is a significant and independent, but modifiable risk to health.

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Appendix A: Hyperinsulinemia: A unifying theory of chronic disease?

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REVIEW

Hyperinsulinemia: A unifying theory of chronic disease?

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ABSTRACT

Globally, there is an increasing prevalence of non-communicable diseases. The morbidity and mortality from these conditions confer a greater economic societal burden. Epidemiological research associates insulin resistance in the etiology of these diseases, but there is limited evidence for the mechanism of damage. Emerging research suggests that hyperinsulinemia, a symptom of insulin resistance, may cause these pathological changes, and therefore be an independent contributor to these diseases. This review shows that hyperinsulinemia, or excessive insulin secretion, should be considered independently to insulin resistance, defined as glucose uptake rate, even though the two conditions are intertwined and will co-exist under normal conditions. Hyperinsulinemia directly and indirectly contributes to a vast array of metabolic diseases including all inflammatory conditions, all vascular diseases, gestational and type 2 diabetes, non-alcoholic fatty liver disease, obesity and certain cancers and dementias. The mechanisms include increased production of: insulin growth factor-1; reactive oxidative species and advanced glycation end-products; and triglyceride and fatty acids. Hyperinsulinemia also directly and indirectly affects many other hormones and cytokine mechanisms including leptin, adiponectin and estrogen. There is limited research standardizing the hyperinsulinemia diagnostic process. Methodological concerns and lack of standardized reference ranges preclude the use of fasting insulin. Most research has also focused on insulin resistance and it is unknown whether these methods translate to hyperinsulinemia.

Keywords: *Hyperinsulinemia, hyperglycemia, type 2 diabetes, insulin resistance, secretagogue, syndrome x*

Introduction

Impaired insulin homeostasis encompasses both hyperinsulinemia and hypoinsulinemia. Although the latter is well recognised as type 1 diabetes, there is little literature on the former, despite being first hypothesised in the early 1920s.[1] Currently, a close approximation to hyperinsulinemia research is that conducted on insulin resistance. Insulin resistance is well-established as underpinning many significant chronic health conditions including type 2 diabetes, metabolic syndrome, cardiovascular disease, some cancers and Alzheimer's disease.[2-5] This insulin resistance is invariably accompanied by increased demands for insulin so that the body can maintain euglycemia. Excess insulin, termed hyperinsulinemia, may be endogenous from bodily compensation, or exogenous via modern medicine. In this paper we contend that hyperinsulinemia, in concert with insulin resistance, should be considered as an important independent health risk. We exclude isolated hyperinsulinemia, such as that caused by an insulinoma.

It is well recognised that earliest detection of any disease state allows for the best possible outcomes. It is agreed that hyperinsulinemia precedes hyperglycemia, by up to 24 years.[3, 4, 6] There is a strong argument that hyperglycemia indicates pancreatic β-cell attrition; essentially end-stage organ damage.[3, 7] We contend that the under-recognition of hyperinsulinemia is an important clinical issue because there are no standard diagnostic reference values, is most accurately diagnosed with dynamic glucose and insulin testing, and has few (pharmaceutical) management options. This review will discuss pathophysiology and diagnosis of hyperinsulinemia.

Hyperinsulinemia was first theorised in 1924[1], but it was not until the 1960's that direct insulin measurements became possible.[8] Since then, there has been a wealth of research in the field of insulin resistance with a small amount of research into hyperinsulinemia. Therefore, we highlight the disease states that are both directly and/or indirectly associated with hyperinsulinemia. We also discuss the availability and limitations of current diagnostic

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methods for hyperinsulinemia and why further investigations are needed.

Methodology

For this narrative review, literature was reviewed on hyperinsulinemia and insulin resistance, targeting full-text English language studies. There was no date criterion. Articles were selected on the basis of having a minimum of both a plausible biological mechanism and established clinical association. Initially, the academic database search included EBSCO, Medline and Google Scholar, using variants of the terms 'hyperinsulinemia', 'insulin resistance', 'metabolic syndrome', and 'syndrome x', individually and conjunction with 'non-communicable disease', 'mechanism', 'atherosclerosis', and 'cardiovascular disease'. As subsequent metabolic diseases and/or mechanisms were eluded to in the initial search, search terms were widened so that no disease state was excluded. Subsequent metabolic diseases included, but were not limited to, conditions such as 'non-alcoholic fatty liver disease', 'cancer', 'dementia'. The final selection of references was based on the authors' judgment of relevance, completeness, and compatibility with clinical, epidemiological, pathological and biochemical criteria.

Hyperinsulinemia

Definition

There is no precise definition of hyperinsulinemia. It is often described as 'more insulin than normal to achieve euglycemia'; essentially the same as insulin resistance. Where a reference range is available, it is normally based on fasting levels and include 5-13 μU/mL[9], ≤ 30 μU/mL[10], and 18-173 pmol/L (3-28 μU/mL)[11] However, there are very few studies where a 'normal level of insulin' is defined as many studies define hyperinsulinemia based on quantiles.[12-14] Few studies have been more specific. Both a fasting serum insulin of ≥12.2 μU/mL in the presence of euglycemia[15] and a range of 8-11 μU/mL 'between meals' and up to 60 μU/mL 'after meals'[16] have been proposed. There are also practical, methodological issues with determining insulin resistance under the World Health Organization (WHO) conditions that will be discussed later in this review.

Etiology

The etiology of hyperinsulinemia is not yet fully elucidated. Although there are several theories, further research will likely show a multimodal pathology. What can be deduced from physiological principles is:

1. Healthy cells are subjected to acute hyperglycemia. 2. Although many cells can absorb glucose without using insulin (GLUT1 transportation) hyperglycemia causes insulin to be released from pancreatic cells to facilitate absorption, especially in muscle and adipose cells (GLUT 4 transportation).[17] 3. Insulin binds to cellular insulin receptors and facilitates translocation of GLUT4 to the cellular surface. During this process the insulin and its receptor are absorbed into the cell to be replaced from the internal pool of insulin receptors.[18] 4. This acute insulin resistance is of no consequence as long as the cell has viable GLUT4 on the cellular surface. However, GLUT4 have a relatively short half-life.[19] 5. If hyperglycemia persists, the pancreas maintains insulin secretion. This may deplete the insulin receptors faster than they can be replaced. 6. During this period where the cells are replacing their insulin receptors, moderately elevated blood glucose levels, (such as that immediately found after a normal meal) may need slightly higher than normal insulin levels to restore normoglycemia. This moderate hyperinsulinemia may delay the return to normal insulin receptor function (acute insulin resistance).[7] This state of insulin resistance due to down-regulated insulin receptors is reversible should the person not be subjected to further episodes of hyperglycemia. It does not matter whether this is via high, but acute, blood glucose elevations, or moderately elevated glucose levels over a prolonged period. 8. Prolonged impaired insulin signaling impedes GLUT4 translocation to the cellular surface thus causing impaired glucose uptake and prolonging hyperglycemia, causing a positive feedback cycle. This will both aggravate and prolong the insulin resistance, potentially turning it from a transitory state to a persistent or chronic state.

The complexity of the insulin receptor regulation, combined with the availability of GLUT4 and factors that influence insulin secretion mean that it is impossible to generalize whether insulin resistance precedes or follows hyperinsulinemia. It is more plausible that different individuals have different triggers in the cycle. These triggers may include genetic factors, excessive carbohydrate, corticosteroids (endogenous or exogenous), free fatty acids, leptin, or certain medications; each of these are discussed below.

Fructose: Fructose is metabolized in liver into ATP and/or triglycerides in a process that is competitive with, and preferential to, glucose. If excessive fructose is consumed, glucose will not be metabolized causing hyperglycemia and subsequent hyperinsulinemia.[20, 21] Excessive fructose also results in hyperuricemia which

is associated with reduced endothelial nitric oxide causing vasoconstriction, endothelial dysfunction and insulin resistance.[21]

Hyperglycemia: Hyperglycemia alone can aggravate insulin resistance.[22] Along with excessive carbohydrate ingestion, other mechanisms for this mechanism include hepatic insulin resistance. Increased plasma insulin slows hepatic gluconeogenesis but this process can be impaired by hepatic insulin resistance leading to peripheral hyperglycemia and further insulin secretion.[23]

Corticosteroids: It is known that corticosteroids, especially endogenous cortisol, cause a down regulation of GLUT-4 receptors, thus preventing glucose uptake and provoking hyperinsulinemia in the presence of hyperglycemia. Long-term courses of exogenous corticosteroids, such as prednisone, are known to cause 'drug-induced' type 2 diabetes, which may resolve after the medication is discontinued. Not every patient on long-term corticosteroids will develop drug-induced diabetes. Therefore, it is plausible that the patient's degree of insulin resistance at baseline influences disease development/progression. Given that stress causes a temporary rise in cortisol levels, it is also plausible that prolonged stress may be another cause of hyperinsulinemia.[24]

Leptin: Appetite control is mediated from the hypothalamus in response to a balance between leptin and insulin controlling neuropeptide Y expression.[25] This balance is believed important to manage caloric intake over longer periods of time when meals can vary in size, frequency and composition. Leptin secretion is slow to change as it is influenced by total body fat mass and total caloric intake, while insulin secretion is highly responsive to food ingestion and will change quickly with every meal. Leptin is also highly influenced by insulin as it is released from fat stores by mechanisms that appear to involve glucose flux.[25] Experimental evidence shows that reducing insulin secretion reduces leptin resistance, suggesting a relationship between hyperinsulinemia and hyperleptinemia.[26] It is not yet clear whether hyperleptinemia is causative of hyperinsulinemia beyond the association of obesity and an increase in free fatty acids.

Medication-induced: There are a number of medications known or suspected to cause hyperinsulinemia and/or contribute to insulin resistance. Exogenous corticosteroids (prednisone) and exogenous insulin and insulin secretagogues (sulphonylureas) have had their mechanisms discussed. Other medications include the antipsychotics (e.g. clozapine), and statins.[27] The mechanisms for these medications causing hyperinsulinemia are currently unknown.

Due to the nature of insulin receptor regulation, it is also plausible that insulin sensitivity of the cells can be restored. This would require the absence of both hyperinsulinemia and hyperglycemia. Case studies indicate that a carbohydrate restricted diet may facilitate this effect.[10]

Overall, it should be recognized that hyperinsulinemia is independent to insulin resistance: Hyperinsulinemia is excessive insulin secretion, while insulin resistance is impaired glucose uptake. This review investigates the both the mechanistic and epidemiological evidence that links hyperinsulinemia to metabolic disease. Although there is good quality research mechanistically linking hyperinsulinemia to subsequent pathologies, there is a paucity of good epidemiological evidence. Given the intertwined nature between insulin resistance and hyperinsulinemia as depicted above, it can be assumed that the majority of people with insulin resistance are also hyperinsulinemic. Therefore, if no epidemiological data was available, this review used epidemiological research based on insulin resistance as a proxy for hyperinsulinemia.

Direct effects of hyperinsulinemia

As shown in Table 1, hyperinsulinemia can be mechanistically and epidemiologically linked to metabolic syndrome, gestational and type 2 diabetes and therefore, cardiovascular and other diseases with an increased prevalence in those with metabolic syndrome.[2-4, 28] It is also an independent risk factor for a number of other diverse conditions including diet-induced obesity, osteoarthritis, certain cancers, especially breast and colon/rectum, and Alzheimer's disease and other dementias.[5, 6, 29-32]

Other conditions that may be associated with hyperinsulinemia, via either epidemiological evidence or potential mechanism of action, include gout, tinnitus, schizophrenia and autism.[33-36] Further research is needed to confirm these associations.

Pathophysiological mechanisms

Hyperinsulinemia affects the body via five main mechanisms: Increased reactive oxidative species and advanced glycation end-products; increased insulin-like growth factor-1 (IGF-1); hyperglycemia; increased fatty acid/triglyceride production; and by affecting different hormones and cytokines.

Reactive oxidative species

Reactive oxygen species is a collective term that includes both oxygen radicals and non-radical

Table 1. Biological systems and disease states affected by hyperinsulinemia, and associated mechanisms of action

Biological System	Disease	Mechanism	Direct or indirect mechanism	References	
				Mechanism of action	Epidemiology
Cancer[†]	Cancer (Breast, ovarian, colon, bladder, pancreas & liver)	Increased insulin-like growth factor IGF-1 enhances cellular growth and proliferation.	Direct	(5, 48)	(29)
		Enhanced glucose uptake and utilization enhances cellular growth and proliferation.	Both	(29)	(29)
		Increased production of reactive oxidative species causes derangement of DNA and enzymes involved with repair mechanisms (enhanced by hyperglycemia).	Indirect	(2, 37, 38)	(2, 37, 38)
		Increased sex-hormone production and decreased sex hormone binding globulin causes increased cellular growth and proliferation (enhanced by obesity).	Direct	(29)	(29)
Circulatory	Atherosclerosis	Arterial wall damage caused by inflammation, increased proliferation and migration of arterial smooth muscle cells. Stimulation of the mitogen-activated protein kinase pathway.	Both	(28, 40)	(28, 47, 63, 64)
		Microvascular disease, including changes to capillary permeability, microaneurysm formation, vasoconstriction and microthrombi.			
	Cardiomyopathy	Increased myocardial fibrosis by increased reactive oxidative species, deranged collagen production.	Both	(65, 66)	(65, 66)
		Diabetic neuropathy causes changes to catecholamines, which further impairs myocardial function.			
	Endothelial dysfunction	Vasoconstriction and pro-atherosclerotic effects from decreased nitric oxide bioavailability and action and increased thromboxane.	Both	(2, 41, 67)	(64)
		Enhanced by increased reactive oxidative species and advanced glycation end-products.			
	Thrombosis	Hyperinsulinemia causes increased fibrinolysis while hyperglycemia causes increased blood coagulability	Indirect	(42)	(64)
		.			
Gastrointestinal	Diabetes: Gestational	Pre-existing insulin resistance and increased demand for insulin.	Direct	(68)	(68)
	Diabetes: Type 2	Prolonged insulin resistance eventuating in beta-cell failure. Down-regulation of glucose transporter-4.	Direct	(3, 69, 70)	(4)
	Hyper- triglyceridemia	Increased triglyceride production.	Direct	(43, 71)	(72)
	Non-alcoholic fatty liver disease	Fatty acid production exceeds distribution capacity. Aggravated by inflammation and oxidative stress.	Direct	(71)	(72)
Endocrine	Chronic inflammation	Stimulation of mitogen-activated protein kinase pathway; glycemic variability; hyperglycemia and/or obesity influences increased cytokine production.	Indirect	(40, 48)	(73)
	Obesity	Decreased lipolysis.	Direct	(74)	(75)
		Lack of appetite suppression.	Direct	(25, 26)	(76)
Nervous	Alzheimer's disease and vascular dementia	Endothelial dysfunction resulting in microvascular disease, metabolic disturbances and neuronal damage.	Direct	(2, 67, 77)	(30, 78, 79)
		Increased blood coagulability and/or fibrinolysis cause multiple thrombotic events.	Both	(42, 80)	

		Changed regulation of beta-amyloid and tau protein (Alzheimer's disease).	Direct	(77, 81)	
		Decreased synaptic plasticity caused by dysregulated PSA-NCAM interactions (Alzheimer's disease).	Direct	(33)	
	Peripheral neuropathy	Increased production of reactive oxidative species and advanced glycation end-products enhanced by hyperglycemia.	Indirect	(2, 41)	(64, 82)
		Insulin resistance in the dorsal root ganglion neurons.	Both	(83)	
	Retinopathy	Hyperglycemia and endothelial dysfunction contribute blood-retinal barrier breakdown. Aggravated by excess advanced glycation end-products.	Direct	(41, 64, 84)	(41, 64, 84)
Skeletal	Osteoporosis	Increased reactive oxidative species and/hyperglycemia cause collagen breakdown, impairs new collagen synthesis and compromises mesenchymal cells.	Indirect	(31)	(31)
Urinary	Nephropathy	Microvascular disease, including changes to capillary permeability, microaneurysm formation, vasoconstriction and microthrombi.	Direct	(67, 85)	(64, 86)
		Increased production of reactive oxidative species and advanced glycation end-products enhanced by hyperglycemia.	Indirect	(41, 87)	

*While cancer is not typically classified as a "biological system", due to its recognition and impact as a key chronic disease, it was decided that it warrants a classification on its own, rather than be integrated into individual biological systems. PSA-NCAM-polysialic acid - neural cell adhesion molecule.

oxidising agents such as hydrogen peroxide.[37] Reactive oxidative species are also produced during, and involved in, many metabolic processes including enzymatic reactions, gene expression and signal transduction.[37] Generally, the actions of intracellular reducing agents such as antioxidants prevent reactive oxidative species-mediated damage. However, a number of factors can contribute to excessive production of reactive oxidative species including excessive calorie consumption and the presence of various pro-inflammatory mediators, including tumor necrosis factor-α.[37] Once produced, reactive oxidative species can interact with numerous cellular components including DNA, lipids, and amino acids. Damage to DNA is likely to be the underlying mechanism for reactive oxidative species being associated with cancer and early aging.[38] Polyunsaturated fatty acids are considered very susceptible to reactive oxidative species damage, triggering lipid peroxidation, which can affect cell membrane fluidity and integrity, potentially being the mechanism for endothelial damage.[37] Amino acids such as cysteine and methionine are very susceptible to reactive oxidative species damage. Changes to these amino acids are implicated in the development of Alzheimer's disease.[39]

Hyperinsulinemia is associated with increased reactive oxidative species, although the exact mechanism is disputed. Hyperinsulinemia is mechanistically linked to excessive serum glucose and free fatty acids. Either substrate can cause increased reactive oxidative species production.[2] Insulin has also been demonstrated to have some inhibitory effects on reactive oxidative species production that may be independent of its effects on glycemia.[40] However, reducing insulin-stimulated nutrient uptake into the cell is also believed to decrease reactive oxidative species production.[2] Further research is required to better understand these mechanisms.

Over-nutrition is also thought to be responsible for the formation of advanced glycation end-products via non-enzymatic glycation and glycooxidation processes.[41] Defective renal excretion of advanced glycation end-products, as seen with diabetic nephropathy, and consumption of exogenous advanced glycation end-products increases plasma advanced glycation end-product levels. Advanced glycation end-products are believed to contribute to changes in the microvascular systems and also promote changes to inflammatory, oxidative and other degenerative processes of various chronic diseases including neuropathies.[41]

Growth factors (IGF, vascular endothelial growth factor)

Insulin, IGF-1 and other substances such as vascular endothelial growth factor (VEGF) can stimulate the growth and division of many cells. Insulin can mediate cellular division but may also stimulate cancer cell proliferation and metastasis.[29] Most importantly, insulin increases the bioavailability of IGF-1, thus insulin is indirectly implicated in all IGF-1 mediated processes. These processes include changes to vascular structures, increases to cellular division and prevention of apoptosis

Hyperglycemia

Hyperglycemia commonly follows hyperinsulinemia[3] but there is little information to suggest whether fasting glucose, peak glucose, or area-under-the curve (AUC) have the most adverse health impact. Cancer cells have a continuously high glucose uptake, which enhances cellular growth and proliferation[20]; hyperglycemia augments this process. Hyperglycemia allows IGF-1 to stimulate vascular smooth muscle proliferation, which is a hall-mark of both cancer and atherosclerosis. Blood coagulability is also increased by hyperglycemia irrespective of insulin levels.[42]

Increased fatty acid and triglyceride production

Hyperinsulinemia influences both free fatty acid and triglyceride production.[43] While the processes that occur during hepatic de novo lipogenesis are not disputed, there is debate as to whether hyperinsulinemia precedes, or are a consequence of fatty liver.[44] Nevertheless, elevated triglyceride levels are recognized to be a key component of metabolic syndrome (Table 1) while fatty liver may be considered a hepatic manifestation of metabolic syndrome and may progress to cirrhosis or hepatocellular cancer.[44] Elevated triglyceride levels may also further impair leptin resistance.[20, 45]

Hormone/cytokine production (sex hormones, inflammation, obesity)

Hyperinsulinemia is involved with adiposity via increased appetite and triglyceride production, thereby increasing adiposity.[46, 47] Adipose tissue is now well-established as an endocrine organ and produces both hormones and cytokines that are used for cellular communication. Hypertrophic adipose tissues activate inflammatory and stress pathways and decreases insulin response. This results in increased cytokine production including TNF-α, vascular endothelial growth factor and leptin, while adiponectin expression is decreased.[48] These actions contribute to decreased glucose and lipid uptake, leading to further reductions to adiponectin secretion and adipogenesis as well as contributing to further insulin resistance. Decreased glucose uptake means there is less glycerol within the adipocyte to esterify free fatty acids, allowing them to infiltrate and accumulate in other tissues.

Adiponectin decreases proliferation of cell types including adipocytes, endothelial cells and cancer cells.[48] The role of leptin is yet to be fully understood, but it is accepted that hyperinsulinemia and hyperleptinemia results in central leptin resistance, and consequent prevention of appetite suppression and promotion of further obesity.[25, 26, 49] Hyperleptinemia is also linked to increased inflammatory cytokines, changes in nitric oxide, and further endothelial injury.[49]

Hyperinsulinemia is also believed to elevate plasminogen activator inhibitor type-1 (PAL-1) levels, with associated increased fibrinolysis and increased risk of thrombosis. When combined with the increased coagulation from hyperglycemia, this may explain why over 80% of people with type 2 diabetes have a thrombotic death.[42]

Diagnosis

Diagnosing hyperinsulinemia is challenging partly because the health effects of insulin resistance and hyperinsulinemia have been conflated. Further challenges arise when interpreting the available literature. As previously discussed, fasting insulin levels have been assessed as a means of diagnosing hyperinsulinemia with differing results. But it is not just the insulin level alone that is problematic. How and when sampling occurs will also cause variation to results. Insulin levels are higher in serum compared to plasma samples meaning that studies reporting serum insulin cannot be compared directly to plasma insulin.[50, 51] Insulin secretion is pulsatile leading to significant levels in plasma insulin in a short space of time. It is recommended that the mean of three samples taken at five minute intervals be used if a fasting insulin level is required[52], however this rarely seem to happen in practice. Single fasting insulin samples can have a coefficient of variation of 25-50%.[53] This variation decreases testing sensitivity and is perhaps why fasting insulin is not recommended to be used clinically.[54]

It is unknown whether insulin resistance testing can be used to diagnose hyperinsulinemia. The gold standard for measuring insulin resistance is the hyperinsulinemic-euglycemic clamp test. The lowest quartile of glucose uptake rate defines insulin resistance for that study population. Figures for this lower quartile have ranged from $< 4.7 mg/kg \cdot min$ to $\leq 6.3 M \cdot mU^{-1} \cdot L^{-1}$, however differences in insulin infusion rates, glucose disposal rate calculations, and background populations under investigation means that there are limits to the generalizability of these results.[15, 55-58] Furthermore, given the complexity of the procedure, the hyperinsulinemic-euglycemic clamp test has little to no clinical application.[15]

A further complication to using the clamp test to assess hyperinsulinemia is that the high dose infusion of insulin will confound any effects of endogenous insulin secretion. As theorized above, the damage associated with hyperinsulinemia is due to the

continuous action of insulin in the tissues. The amount of insulin normally present in the tissues cannot be measured during the clamp process. It is unknown whether glucose uptake rates correlate with insulin secretion.

A number of tests have been developed that are validated against the hyperinsulinemic-euglycemic clamp that has more clinical applicability. Those based on fasting insulin include homeostatic model assessment (HOMA or HOMA2), McAuley Index, and the quantitative insulin sensitivity check index (QUICKI).[15, 56, 59] Although HOMA has since been refined to the HOMA2 model, both are modelled on the combination of fasting insulin to fasting glucose. The original HOMA has a 89% sensitivity and 67% specificity compared to hyperinsulinemia-euglycemic clamp.[57] The McAuley index is calculated from fasting insulin and fasting triglyceride levels with 61% sensitivity and 85% specificity.[15]

Another insulin resistance test, the oral glucose sensitivity index (OGIS), is modelled on the results derived from an oral glucose tolerance test.[56] OGIS uses both blood insulin and glucose levels at baseline, 120 min and 180 min. A spreadsheet is recommended for the calculations (available from http://webmet.pd.cnr.it/ogis/download.php). The OGIS is validated against the hyperinsulinemic-euglycemic clamp assessments for insulin resistance, but as previously stated, the generalizability of clamps is limited.

Both the OGIS and tests based on fasting insulin levels have more clinical applicability for assessing insulin resistance compared to the hyperinsulinemic-euglycemic clamp test. However, insulin resistance testing has never translated to improvements in disease risk calculations. The WHO definition for insulin resistance means that one in four people would be diagnosed with insulin resistance; a figure that may be unrelated to their actual health risks.[60] Analysis from the Women's Health Initiative Biomarkers study showed that although HOMA-IR had a positive association with cardiovascular risk, this was became non-significant after adjusting for other risk factors such as HDL cholesterol.[61] There is an argument that HOMA-IR should be used in combination with HOMA-%B for assessing insulin resistance.[52]

Emerging research now suggests that insulin response patterns following an oral glucose load may determine hyperinsulinemic status. Kraft[7,10] demonstrated the variability of insulin response to a 100 g glucose load over 3-5 hours, especially with respect to timing and magnitude of the insulin peak and rate of response decline. Five main insulin response patterns are clearly identifiable, with pattern I being considered normal insulin tolerance. From this research Kraft concluded that the most accurate means of assessing hyperinsulinemia was a 3-hour oral glucose tolerance test with insulin levels assessed at baseline, 30, 60, 120, and, at minimum, 180 minutes but 240 and 300 minute insulin levels could also be considered. This study was cross-sectional and there are no long-term outcomes.

Hayashi and colleagues[62] have shown that the insulinemic pattern produced from sampling every 30 minutes during a 2-hour OGTT can predict the development of type 2 diabetes. An insulin peak delayed beyond 60 minutes being associated with poorer health is common to both Kraft and Hayashi patterns. Further research is required to understand how to apply these patterns to clinical practice.

Collectively these studies show that there is a paucity of research for diagnosing hyperinsulinemia. Most studies focus on insulin resistance testing, but it remains unknown whether insulin resistance correlates with insulin secretion.

Concluding remarks

This review clearly demonstrates that not only is hyperinsulinemia involved with the etiology of all of the symptoms of metabolic syndrome, it is also implicated in many other conditions; some of which have previously been considered to be idiopathic, such as tinnitus. This raises many questions with both clinical and research implications. Firstly, what is the prevalence of hyperinsulinemia? Given its association with metabolic syndrome and fatty liver disease, this warrants investigation. Could early detection and careful management of hyperinsulinemia decrease the need for medical interventions later in life? Would managing hyperinsulinemia improve to both quantity and quality of life? Yet there are currently too many questions regarding diagnosis. A reliable and repeatable result when sampling insulin is still a challenging task. There is no agreed upon reference range, and there are only associations between quantiles and ongoing disease risk. Insulin response patterning may answer some of these questions, but patterning requires more resources than a fasting level. Given the global concerns about the 'epidemic' of metabolic diseases, this research needs to be urgently addressed.

Conflict of interest

None Declared.

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Appendix B: HDEC approval for analysing the Kraft database

Health and Disability Ethics Committees

Health and Disability Ethics Committees
1 The Terrace
C/- MEDSAFE, Level 6, Deloitte House
10 Brandon Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

30 October 2013

Mrs Catherine Crofts
Human Potential Centre
Auckland University of Technology
Private Bag 92006
Auckland 1142

Dear Mrs Crofts

Re:	Ethics ref:	13/CEN/166
	Study title:	Comparing Kraft pattern methodology with modern diagnostic algorithms to confirm best practice for diagnosing hyperinsulinaemia.

I am pleased to advise that this application has been *approved* by the Central Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Central Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Participant access to ACC

The Central Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Mrs Helen Walker
Chairperson
Central Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	*Version*	*Date*
CV for CI: Academic CV for Catherine Crofts	2	03 October 2013
Protocol: Protocol to analyse Kraft's data	1	22 October 2013
Peer review letter.	1	22 October 2013
Evidence of scientific review: Evidence of peer review Caryn and Grant	1	22 October 2013
Application	1	23 October 2013

Appendix B
Statement of compliance and list of members

Statement of compliance

The Central Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008712) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Mrs Helen Walker	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Angela Ballantyne	Lay (ethical/moral reasoning)	01/07/2012	01/07/2015
Mr Paul Barnett	Lay (the law)	01/07/2012	01/07/2014
Mrs Gael Donoghue	Non-lay (health/disability service provision)	01/07/2012	01/07/2014
Mrs Sandy Gill	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Patries Herst	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Dean Quinn	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Lynne Russell	Non-lay (observational studies)	01/07/2012	01/07/2014

http://www.ethics.health.govt.nz

Appendix C: AUTEC approval for analysing the Kraft database

AUT UNIVERSITY | AUTEC SECRETARIAT

20 November 2013

Grant Schofield
Faculty of Health and Environmental Sciences

Dear Grant

Re: Ethics Application: **13/337 Comparing Kraft pattern methodology with modern diagnostic algorithms to confirm best practice for diagnosing hyperinsulinaemia.**

Thank you for submitting your application for ethical review to the Auckland University of Technology Ethics Committee (AUTEC. I am pleased to confirm that the Chair and I have approved your ethics application for three years until 14 October 2016.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 18 November 2016;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 18 November 2016 or on completion of the project;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within their.

To enable us to provide you with efficient service, we ask that you use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

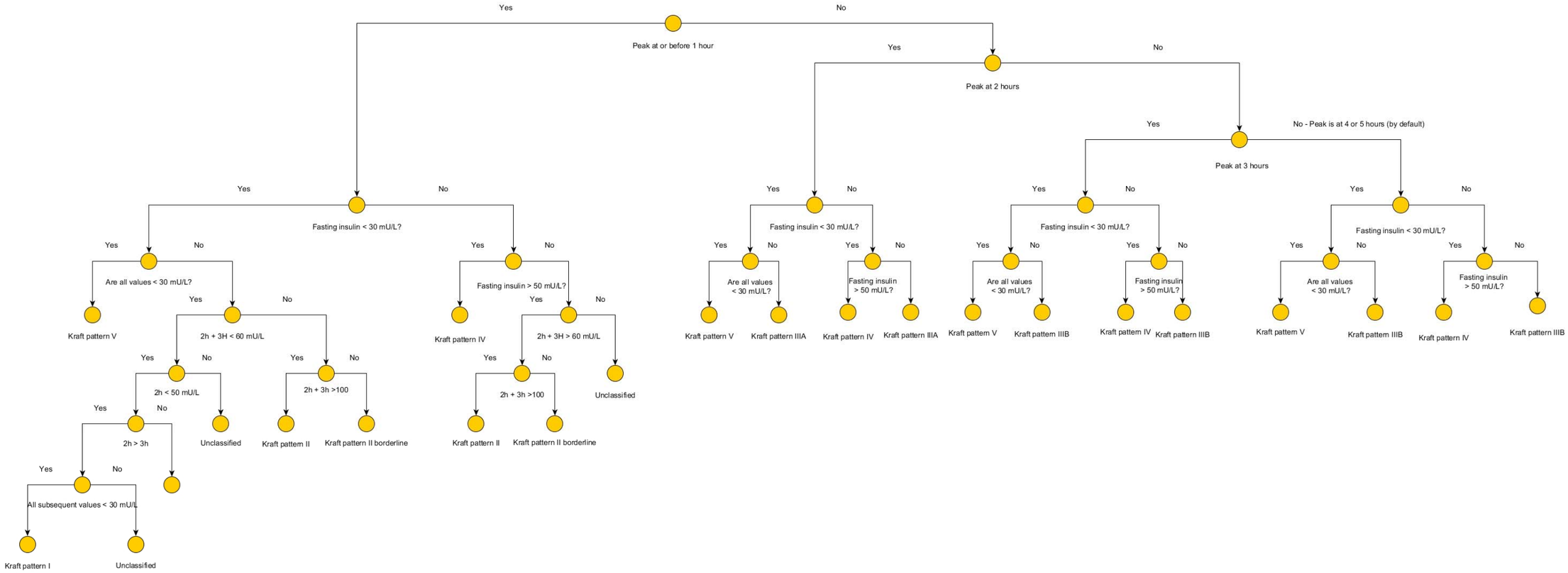
All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee

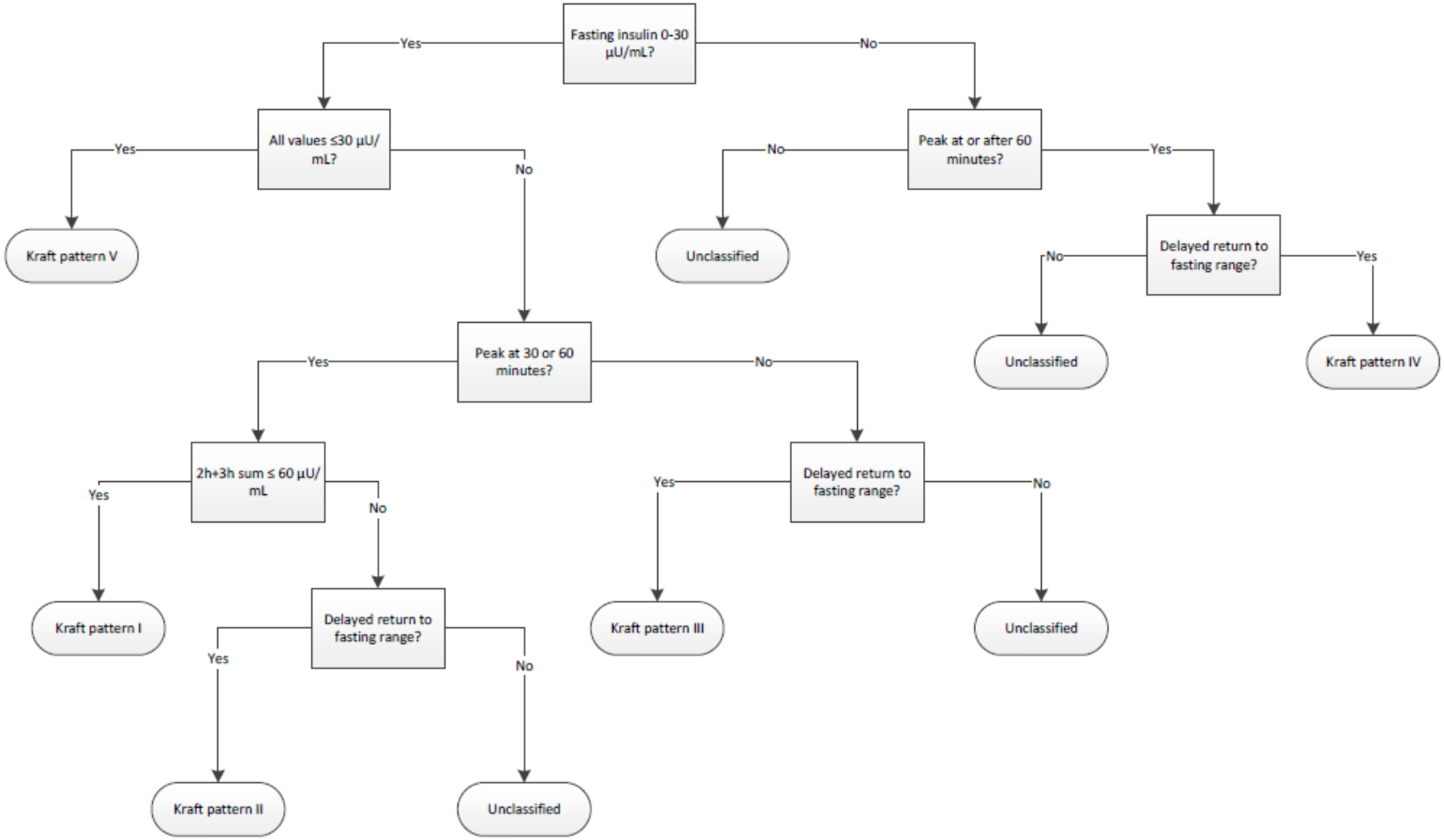
Cc: Catherine Crofts ccrofts@aut.ac.nz

Auckland University of Technology Ethics Committee
WA505D Level 5 WA Building City Campus
Private Bag 92006 Auckland 1142 Ph: +64-9-921-9999 ext 8316 email ethics@aut.ac.nz

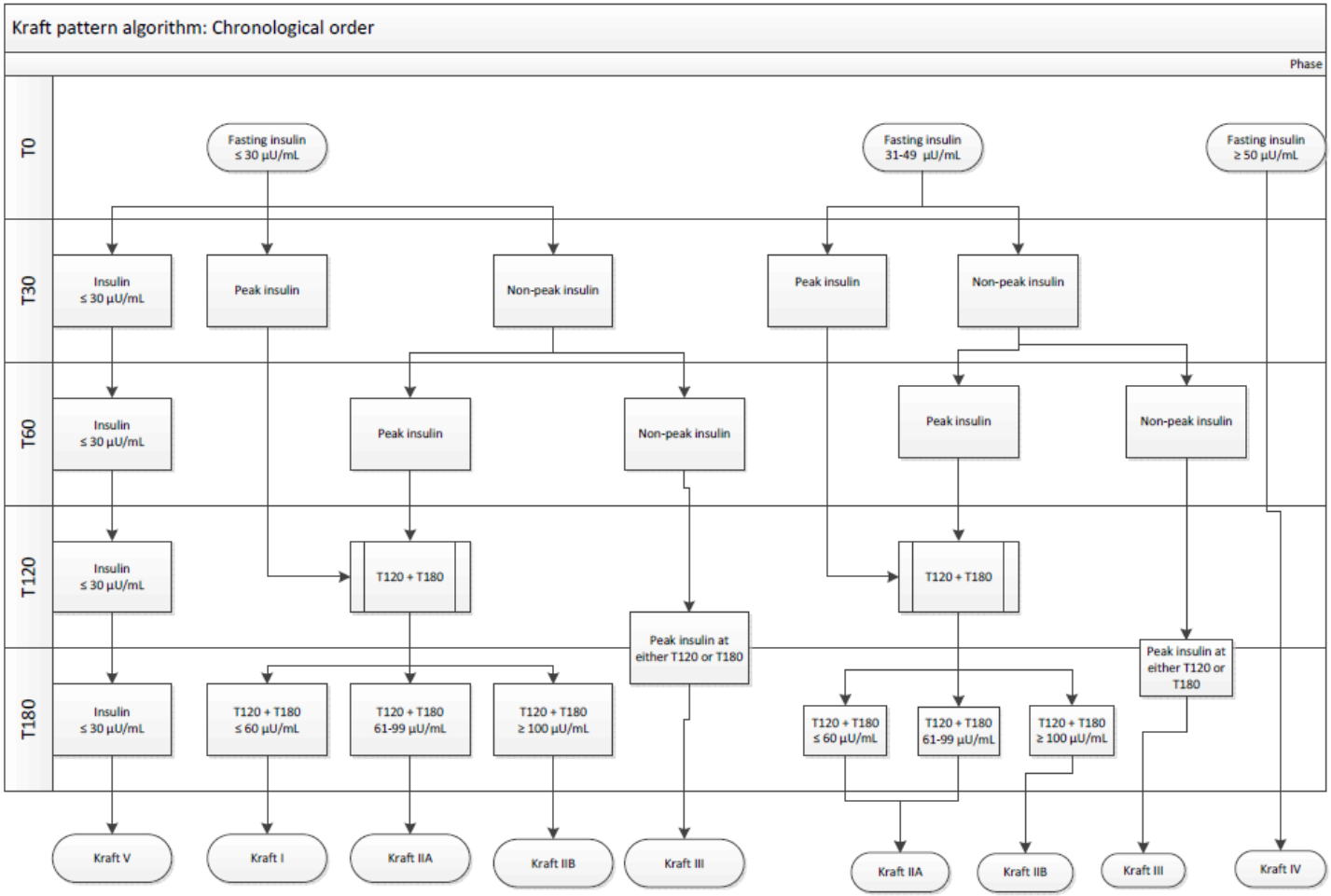
Appendix D: Kraft 1975 classification tree



Appendix E: Kraft 2008 classification tree



Appendix F: Kraft pattern algorithm: Chronological order



Appendix G: AUTEC approval for assessing the repeatability of insulin response patterns

AUT UNIVERSITY | AUTEC SECRETARIAT

16 December 2014

Grant Schofield
Faculty of Health and Environmental Sciences

Dear Grant

Re Ethics Application: **14/363 Test-retest reliability of Kraft patterns for testing hyperinsulinaemia (high insulin levels).**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 15 December 2017.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 15 December 2017;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 15 December 2017 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply there.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee

Cc: Catherine Crofts ccrofts@aut.ac.nz

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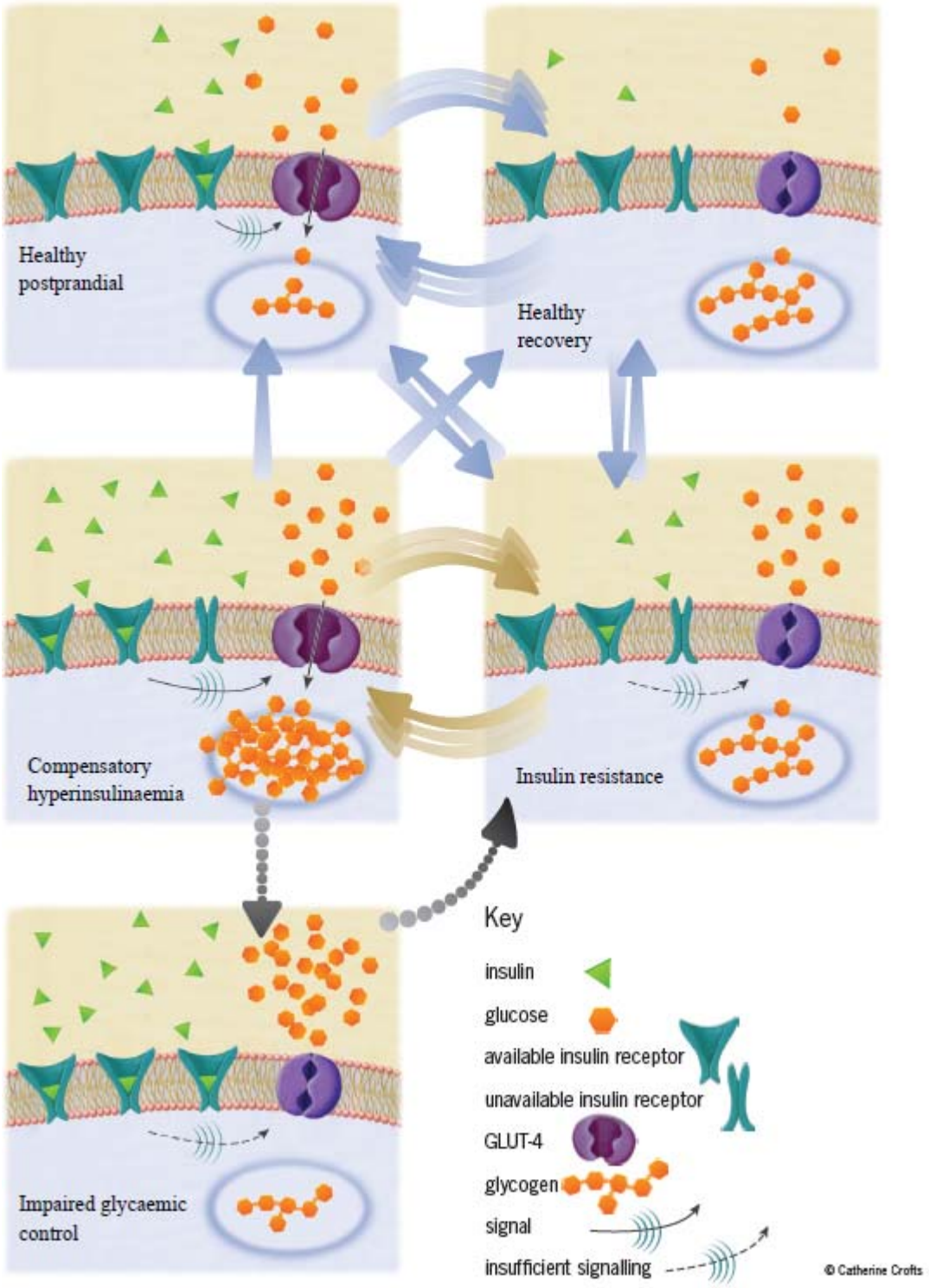
Appendix H: Transitions between different states in the hyperinsulinaemia spectrum from healthy to impaired glycaemic control.



Healthy post-prandial: Elevated blood glucose stimulates insulin release, which binds to insulin receptors signalling GLUT4 to up-regulate and transport glucose into the cell to be used for immediate energy needs or stored as glycogen.

Healthy recovery: Blood glucose and insulin are at basal levels. Used insulin receptors require recovery time (estimated at two to six hours) to regain availability. GLUT4 are down-regulated and require further insulin stimulus to allow glucose transport.

Insulin resistance: The normal amount of insulin does not up-regulate GLUT4 and allow glucose transport. This may be due to down-regulated GLUT4 or insufficient available insulin receptors.

Compensatory hyperinsulinaemia: Sustained blood glucose elevations during insulin resistance cause insulin hyper-secretion (hyperinsulinaemia) in order to obtain sufficient signalling from the insulin receptors to up-regulate GLUT4 and transport glucose to restore euglycaemia. If the cell has sufficient glycogen, excess glucose will be stored as fat (not shown).

Impaired glycaemic control: Despite hyperinsulinaemia, insulin receptors cannot maintain sufficient GLUT4 up-regulation to maintain euglycaemia. Fasting and/or post-prandial hyperglycaemia develops. Hyperinsulinaemia inhibits lipolysis so only glycogen can be metabolised for energy.

Cells will transition back from 'Healthy recovery' to a pre-prandial state ready to receive a glucose load if given sufficient time without additional glucose stimulus. If healthy cells are exposed to certain stimuli, including insufficient recovery time, or are subjected to excessive glucose or other factors that down-regulate either insulin receptors or GLUT4, they may become Insulin resistant. Insulin resistant or hyperinsulinaemic cells may be able to return to a healthy state assuming the initial stimulus is removed and they have sufficient recovery time (without glucose stimulus), and/or their GLUT4 are up-regulated. As the cycle between Insulin resistance and Hyperinsulinaemia is a positive feedback loop, the longer the cell cycles between these two states, a more extensive recovery period may be required. In many cases, a full return to a healthy state may not be possible, but further damage may be able to be mitigated. Prolonged time in the Insulin resistant - Hyperinsulinaemia cycle may result in decreased insulin receptor and/or GLUT4 capacity to the extent that hyperglycaemia is sustained, resulting in impaired glycaemic control. Medical intervention will be required if lifestyle changes are insufficient to maintain euglycaemia, but as later discussed, this is unlikely to resolve hyperinsulinaemia.