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Protocol

GIL-1665

Acute effect of Axulin on Blood Glucose and Serum Insulin Responses in Healthy Lean and Overweight Humans.

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INTRODUCTION

After consumption of a meal, pancreatic secretions of various digestive enzymes results in the breakdown of carbohydrates into monosaccharides including glucose.[1] These sugars are subsequently absorbed through the intestinal lumen, resulting in an increased plasma glucose concentration.[1] In response to high glucose levels, pancreatic beta-cells are stimulated to release the hormone insulin which circulates through the bloodstream and binds to insulin-responsive cells including adipocytes (fat tissue), myocytes (muscle tissue), and hepatocytes (liver).[1] The resulting insulin-mediated signaling cascade initiates intracellular glucose uptake within peripheral tissues leading to a corresponding decrease in circulating plasma glucose.[1]

In insulin responsive cells glucose uptake stimulation begins after the binding of insulin to Insulin Receptors (IR), which are found on the membrane surface of cells in insulin responsive tissues such as fat, muscle and liver. The IR consists of an extracellular domain which binds to insulin, and an intracellular domain that has a protein tyrosine kinase activity.[2] The binding of Insulin to the IR initiates a series of auto-phosphorylation events within the protein kinase domain that permit interaction and phosphorylation of downstream signaling proteins in the cell that mediate the cellular response to insulin.[3-4] The resulting signaling complex includes proteins in the Insulin Receptor Substrate (IRS) family known as IRS-1 and IRS-2.[5-8] These key targets of the insulin signaling pathway link IR activation to downstream signaling cascades that mediate intracellular processes including GLUT4 mediated glucose uptake.[9-16]

Pre-diabetes and Type II diabetes involve an impaired post-receptor response to insulin that hinders the glucose uptake response after meal consumption.[17-19] Chronic hyperglycemia and the resulting compensatory hyperinsulinemia promote a cohort of acute and chronic sequelae including cardiovascular disease, liver complications, central nervous system degeneration and hyperglycemic osmotic stress.[20-23] Axulin is a natural health product consisting of a mixture of extracts from herbs and vegetables present in normal diets which was identified by screening more than 100,000 compounds and extracts in a patented cell-culture based assay system targeting the IRS proteins. In vitro, Axulin has marked effects on the IRS-2 branch of the insulin signaling cascade to enhance downstream insulin signaling. Axulin has been shown in animal models to increase glucose uptake in peripheral tissues and decrease circulating blood glucose and triglyceride concentrations. Regular supplementation of the diet with Axulin would be expected to reduce the incidence of associated pre-diabetic and diabetic complications, resulting in an increased quality of life for patients without resorting to current anti-diabetic prescription drugs such as metformin and others that may have substantial unwanted side effects in patients.

HYPOTHESES

Axulin will reduce postprandial glucose and insulin responses in a dose-dependent fashion in healthy subjects without diabetes. The reduction in glucose and insulin will be relatively greater in insulin-resistant than insulin-sensitive subjects.

OBJECTIVES

The primary objective of this study is to determine the dose of Axulin which will reduce the incremental area under the blood glucose response curve (AUC) elicited by 75g oral glucose in healthy subjects by ≥33%.

The secondary objectives are to determine the dose-response effects of Axulin on:

- blood glucose and serum insulin AUC
- % reduction of insulin AUC
- the blood glucose and serum insulin increments at 15, 30, 45, 60, 90 and 120min
- insulinogen index
- Matsuda insulin sensitivity index
- ISSI-2 index of beta-cell function
- incremental blood lipid response at 120min
- plasma triglyceride response
- plasma axulin response

The other objectives are to determine whether the effects of Axulin on glucose, insulin and triglyceride responses differ in lean vs overweight subjects and to determine whether the effects of Axulin on postprandial responses are similar when administered in capsule versus tablet form.

SUBJECTS

Inclusion criteria

Subjects will be males or non-pregnant, non-lactating females aged 18-60 years who have no history of diabetes and are in good health. Lean subjects will have a BMI >18.5 and <25.0kg/m², overweight subjects will have a BMI ≥25.0 and <35.0kg/m². Subjects taking stable doses (for at least 4 weeks of signing the consent form) of the birth control pill, thyroxin replacement therapy, statins, fibrates, cholesterol absorption inhibitors, anti-hypertensive medications, asprin, non-steroidal anti-inflammatories and/or mild anxiolytics or sedatives can be included.

Female subjects of child-bearing potential must, for the duration of the study, be using one of the following birth control methods: total abstinence, a hormonal birth control method (oral, injectable, transdermal, or intra-vaginal), intrauterine device, or confirmed successful vasectomy of partner.

Exclusion criteria

- Fasting serum glucose >6.9mmol/L (124mg/dL)
- HbA1c >6.4%
- Fasting serum triglycerides >4.5 mmol/L (399 mg/dL)
- Fasting LDL cholesterol >4.99 mmol/L (192 mg/dL)
- Blood pressure >149 systolic or >89 diastolic
- Serum creatinine, or aspartate- or alanine transaminases >1.2 times upper limit of normal
- White cell count, red blood cell count, hemoglobin or hematocrit outside normal range
- Hospitalization for surgery or a medical condition within 3 months of signing the consent form

- Use of any drug to treat diabetes
- Use of medications other than those listed above or the presence of any condition which might, in the opinion of Dr. Wolever, either: 1) make participation dangerous to the subject or to others, or 2) affect the results
- Allergy or sensitivity to tarragon, chromium, escarole, lettuce, microcrystalline cellulose, inulin, food colouring (FD&C Yellow#5 and Blue#1) or vegetable-based capsules (silicon dioxide, titanium dioxide, hydroxypropylmethylcellulose)

Number of subjects

The primary endpoint is the % reduction in 2-hour incremental AUC for glucose. Using the t-distribution with 2-tailed $p<0.05$ and assuming an average CV of within-individual variation of 2-hour incremental AUC values of 25%, n=20 subjects (10 lean and 10 overweight) has 80% power to detect a 17% difference in AUC, 90% power to detect a difference of 19% and 99% power to detect a difference of 26%. Within each subject group (n=10) there is 80% power to detect a difference in AUC of 25%, and 80% power to detect a 33% difference between the responses of lean and overweight subjects. To allow for 10% dropout, we will recruit n=11 lean and n=11 overweight subjects.

Subject Recruitment

Participants will be recruited from the pool of participants at GI Laboratories who have indicated that they are willing to be contacted and asked if they wish to participate in new studies. If an insufficient number of subjects can be recruited, then a newspaper advertisement will be used to recruit new subjects.

Selection of participants

Participants willing to be considered will be invited to come to the research centre to have the study procedures explained to them and be given a copy of the consent form which they may either sign then, take away to sign at a later date, or decline to participate. Participants will be encouraged to ask any questions they may have and not to sign the consent form until all of their questions have been answered to their satisfaction. Those who consent to participate will come to the research centre for a pre-selection visit when subject eligibility will be determined. During this visit, the 10-14hr overnight fasted participants will complete a questionnaire (Appendix 1) to assess medical history, and lifestyle factors, have height, weight, waist circumference and blood pressure measured, and have a blood sample drawn for measurement of glucose, HbA1c, lipids, creatinine, AST, ALT and complete blood count. Females will have a pregnancy test.

PROCEDURES

The study will have a randomized block design with repeated measures. One block, which will be double-blinded, will test the effect of Axulin capsules; the other block, which will be open label, will test the effect of Axulin tablets. Within each participant, the order of the blocks will be randomized and within each block the order of the treatments will be randomized.

Eligible participants will be instructed to maintain stable dietary and exercise habits and for the duration of the study, to avoid vigorous physical activity for 24hr before each visit and to and consume their normal dinner the night before each visit. At each visit current use of medications and natural health products participants will be reviewed with each participant and any changes will be discussed with the investigator. Female participants will be advised that if they become pregnant during the study they are to contact the investigators and discontinue their participation immediately.

Each subject will be studied on 6 separate days with at least a 3-day interval between tests. On each test day, subjects will come to GI Labs (20 Victoria Street, Toronto) in the morning after a 10-14h overnight fast. On each test occasion the subject will be weighed, and give fasting finger-prick and venous blood samples. Blood from a forearm vein will be collected into a 6ml EDTA tube (Vacutainer, BD Canada, Mississauga, ON). Finger-prick blood samples will be collected into 2 separate vials: one (2-3 drops blood into a fluoro-oxalate tube) for glucose analysis and the other (6-8 drops of blood into an 0.3ml Microvette with clot activator, Sarsted, Germany) for insulin. After the fasting sample the subject will take 12 capsules or 2 tablets with a drink of 250ml water. Subjects will rest for 3-10min and have their blood pressure measured; 40min after swallowing the first capsule or tablet a finger-prick blood sample (2-3 drops for glucose and 6-8 drops for insulin analysis) will be collected and then subjects will consume 82.5g dextrose (containing 75g anhydrous glucose) dissolved in 300ml water within 5min plus a drink of 150ml water within 10min of starting the glucose drink. Further finger-prick blood samples for glucose (2-3 drops) and insulin (6-8 drops) will be obtained at 15, 30, 45, 60, 90 and 120 min after starting to drink the glucose solution. Venous blood samples (7ml) will be collected after the 60 and 120 min finger-prick. During the test subjects will remain seated quietly except for trips to the washroom if necessary. After the last blood sample subjects will be offered a snack and be free to leave.

Treatments

The 6 treatments administered will consist of the following:

- 12 placebo capsules wit 250ml water (0g Axulin)
- 250ml water alone (0g Axulin

- 4 placebo and 8 Axulin capsules (2g Axulin)
- 16 Axulin capsules (4g Axulin)
- 2 Axulin tablets (2g Axulin)
- 4 Axulin tablets (4g Axulin)

Each placebo capsule contains 250mg inulin, each identical-looking Axulin capsule contains 250mg Axulin. It is anticipated that Axulin will be sold in tablets because 1g can be compacted into a tablet without loss of biological activity, but, because Axulin is a low-density powder, only 400mg can be placed in a capsule of similar size. We will use capsules in this study because it is more economical to produce the relatively small number of identical-looking placebo capsules required as compared to producing placebo tablets. To obtain doses in 1g increments, we will use smaller capsules containing 250mg each. The rationale for including tablets in the study is that Axulin in tablet form might be more slowly bioavailable than when encapsulated; thus, it is

important to determine whether the effects of Axulin tablets on postprandial responses is similar to the same dose taken in capsules.

Blinding

The number of Placebo and Active Axulin capsules and tablets required for the study will be provided to GI Labs. A person not involved in any other aspect of the study will make up 6 small zip-lock plastic bags for each subject labelled with a subject number and visit number and containing the treatment for vists 1 through 6 (the bag for the water alone treatment will be empty). The treatments will be randomized as shown in Table 1. The order of the blocks and the order of the treatments within each block were randomized using the @rand() function of Excel with 2 blocks of 6 created for lean subjects and 2 blocks for overweight subjects; the 2 extra orders are for use in case of drop-outs so that at least n=20 subjects complete the study (see Table 1). The person labeling and filling the bags with the treatments will assign the treatments to the codes and place the treatment assignments in a sealed opaque envelope kept in an accessible location. The envelope will be opened only after the study has been completed and the database locked, or in case of emergency if required for participant safety.

Table 1: Randomization Scheme

ID	Group	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
1	Lean	A	B	C	E	D	F
2	Lean	B	C	A	E	F	D
3	Lean	F	D	E	C	A	B
4	Lean	B	A	C	F	E	D
5	Lean	D	E	F	A	C	B
6	Lean	D	F	E	C	B	A
7	Lean	F	D	E	A	C	B
8	Lean	C	B	A	D	E	F
9	Lean	D	F	E	B	C	A
10	Lean	C	A	B	E	D	F
11	Lean	B	A	C	E	F	D
12	Lean	F	E	D	A	B	C
21	Overweight	D	E	F	C	B	A
22	Overweight	F	D	E	B	A	C
23	Overweight	A	B	C	E	D	F
24	Overweight	A	C	B	F	E	D
25	Overweight	B	C	A	E	F	D
26	Overweight	C	A	B	D	F	E

27	Overweight	B	C	A	F	D	E
28	Overweight	D	F	E	B	A	C
29	Overweight	D	E	F	C	B	A
30	Overweight	F	E	D	A	B	C
31	Overweight	A	C	B	E	F	D
32	Overweight	E	D	F	C	A	B

Instructions: please assign capsules to codes A, B or C, and tablets or empty bag to codes D, E or F. Place treatment assignments into a sealed opaque envelope and keep the envelope in an accessible location. Treaments are as follows:

BLOCK 1 (A, B or C)

- Placebo 1; 16 Placebo capsules
- 2g Axulin caps: 4 Placebo capsules plus 8 Axulin capsules
- 4g Axulin caps: 16 Axulin capsules

BLOCK 2 (D, E or F)

- empty bag
- 2g Axulin tablets: 2 Axulin tablets
- 4g Axulin tablets: 4 Axulin tablets

Biochemical Analysis

Finger-prick blood samples for glucose analysis will be placed in the refrigerator immediately after being obtained and at the end of the test placed in a -20˚C freezer until analysis which will be performed within 5 days. Glucose analysis is done using an YSI model 2300 STAT analyzer (Yellow Springs, OH). The microvette tubes used to collect blood for insulin analysis will be centrifuged at 3622×g for 2 min and the serum transferred to labeled polypropylene tubes and stored at -20˚C prior to analysis of insulin using the Human Insulin EIA Kit (Alpco Diagnostics). Venous blood samples will be stored at 4ºC until the end of the study after which they will be centrifuged at ~1400×g for 10 min and the plasma removed and separated into 2×1ml aliquots; 1 for analysis of triglycerides at St. Michael's Hospital biochemistry laboratory and the other for measurement of the active ingredient in Axulin by liquid chromatrography/mass spectrometry.

Statistical Analysis

Incremental areas under the blood glucose, insulin and triglyceride response curves (AUC), ignoring area below fasting, will be calculated. Blood glucose and insulin concentrations and AUC values will be analyzed using repeated measures ANOVA examining for the main effects of dose, form (tablets vs capsules) and subject group (lean vs overweight) and their interactions. After demonstration of significant heterogeneity, differences between individual means will be assessed using Tukey's test to adjust for multiple comparisons.

Informed Consent

The GI Testing protocol has been approved by the Western Institutional Review Board® which meets all requirements of the US Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS), the Canadian Health Protection Branch (HPB), Canadian

Institutes for Health Research (CIHR) and the European Community Guidelines. Informed consent is obtained from all subjects for each series of tests they participate in.

Participant Withdrawal Criteria
The Investigator may elect to withdraw the participant from the study or the participant may withdraw from the study at any time. Reasons for withdrawing include, but are not limited to:

- Development of an exclusion criterion during the course of the study
- Study product never consumed
- Participant was lost to follow-up
- Participant did not follow study procedures
- Participant elected to withdraw from the study for any reason
- Investigator elected to remove participant from the study for any reason
- Subject takes longer than the specified time to complete the study

In the case of premature withdrawal, all planned assessments for the end of the study visit will be completed and the sponsor will be informed as soon as possible. The subject's Case Report Form ("CRF") will be completed including the final assessment. The date and main reason for the premature withdrawal will be clearly documented in the subject's file and written down on the CRF

ADVERSE EVENT REPORTING
An adverse event (AE) is any symptom, injury, illness, or medical/surgical procedure whether planned or not or whether associated with the study foods or not. AEs will be assessed at each visit and recorded in the Case Report Forms. AEs will be evaluated for duration, severity, seriousness, and causal relationship to the food items provided in the study by the study physician who will decide if any action is required and provide referral, treatment, and/or follow-up as appropriate until resolved, judged to no longer be clinically significant, or if a chronic condition, until fully characterized. Research staff will record the final outcome and resolution date of the event wherever possible. The action taken and the outcome will be recorded in the Case Report Form.

It is anticipated that participants enrolled in the study will be clinically managed without the need to add medications or to change the dosage(s) assigned to their current medications. In the event that additional medication is required, or an increase in their medication dosage is warranted, these changes will be recorded as adverse events.

Investigators are required to report adverse events that fit the following criteria within 10 working days of the time the investigator becomes aware of them:

- Event is **Unexpected** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, or the

Investigator Brochure; and (b) the characteristics of the subject population being studied.

- **Related or possibly related** to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research **places subjects or others at a greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

If the adverse event is clearly not related to the study drug, device, procedures, or washout process, it would not represent a risk to other subjects in the research or a "problem" for the study and, therefore, does not have to be reported to WIRB.

The Principal Investigator, Dr. Thomas Wolever, will be informed within 48 hrs of the nature of the reportable AE and the treatment allocation of the participant in whom it occurred by sending a copy of the AE report via email (thomas.wolever@utoronto.ca). Dr. Wolever will track all reportable AEs and notify the sponsors if they occur.

In addition to the above, all adverse events will be reported to Health Canada. All serious adverse events (any adverse event that results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect) will be reported to Health Canada, the Sponsor and the IRB within 24hr of their ascertainment.

Risks

Healthy volunteers have been found to be able to tolerate doses of up to 60g of resistant maltodextrins with few symptoms (Storey et al. 2007). Since the test meals used in this study contain a maximum of 12g resistant starch, we do not expect subjects to experience any GI symptoms, or if symptoms do occur, they would be very mild (eg. mild flatulence). However, there may be rare occurrences of study product intolerance, as manifested by severe gastrointestinal symptoms (such as crampy gas or watery stools), eczema, rashes, or other signs of allergy, or infection.

Dispensing

It is the responsibility of the Investigator or his designee to ensure that study product is only provided to study participants.

All study products will be consumed during test sessions at Glycemic Index Laboratories and will not be dispensed to subjects to consume outside of the facility. The specific serving size for each product will be provided to the Investigator along with instructions for administration of each product.

Source documents and archiving

All documents relating to the study will be kept by the investigator: the original informed consent form, source documents, copy of the CRF, copy of the accountability of products

administered, copy of the WIRB (ethics committee) approval and correspondence with the sponsor. The investigator will authorize direct access to the source documents during monitoring. As required by Canadian law, documents will be kept by the investigator for at least 25 years after the closure of the study.

BUDGET

Expense Category	**Amount ($CDN)**
Screening	8,450
Hospital Lab fees	3,475
WIRB submission	4,375
Subject payments	9,840
Supplies	14,370
Staff	
Study coordinators: 144hr @ $45	6,480
Junior Staff: 138hr @ $30	4,140
Lab technicians: 439hr @ $30	13,170
Data analysis	4,000
Protocol/Report /Manuscript	5,800
Space utilization	31,400
TOTAL	105,500

References

1. Kasper D, Fauci A, Hauser S, Longo D, Jamerson JL, Loscalzo J. “Harrison's principles of internal medicine” New York: McGraw-Hill Medical, 2008.

2. Ward CW, Lawrence MC, Streltsov VA, Adams TE, McKern NM. The insulin and EGF receptor structures: new insights into ligand-induced receptor activation. Trends Biochem Sci. 2007 Mar;32(3):129-37.

3. White MF, Shoelson SE, Keutmann H, Kahn CR. A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. J Biol Chem. 1988 Feb 25;263(6):2969-80.

4. Till JH, Ablooglu AJ, Frankel M, Bishop SM, Kohanski RA, Hubbard SR. Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: insights into kinase mechanism. J Biol Chem. 2001 Mar 30;276(13):10049-55

5. White MF. Insulin signaling in health and disease. Science 2003 Dec 5;302(5651):1710-1.

6. Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. Trends Endocrinol Metab. 2010 Oct;21(10):589-98.

7. Yenush L, Zanella C, Uchida T, Bernal D, White MF. The pleckstrin homology and phosphotyrosine binding domains of insulin receptor substrate 1 mediate inhibition of apoptosis by insulin. Mol Cell Biol. 1998 Nov;18(11):6784-94.

8. White MF, Kahn CR. The insulin signaling system. J Biol Chem. 1994;269(1):1-4

9. Yecies JL, Zhang HH, Menon S, Liu S, Yecies D, Lipovsky AI, Gorgun C, Kwiatkowski DJ, Hotamisligil GS, Lee CH, Manning BD. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab. 2011 Jul 6;14(1):21-32.

10. White MF, Copps KD, Ozcan U, Tseng YD. The Molecular Basis of insulin action. In: Jameson JL, DeGroot LJ, editors. Endocrinology. 6th ed. Philadelphia: Elsevier; 2010. p. 636-59.

11. White MF. Regulating insulin signaling and beta-cell function through IRS proteins. Can J Physiol Pharmacol. 2006 Jul;84(7):725-37.

12. Taniguchi CM, Ueki K, Kahn CR. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. J Clin Invest. 2005 Mar;115(3):718-27.

13. Dong X, Park S, Lin X, Copps K, Yi X, White MF. Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. J Clin Invest. 2006 Jan;116(1):101-14

14. Kubota N, Kubota T, Itoh S, Kumagai H, Kozono H, Takamoto I, Mineyama T, Ogata H, Tokuyama K, Ohsugi M, Sasako T, Moroi M, Sugi K, Kakuta S, Iwakura Y, Noda T, Ohnishi S, Nagai R, Tobe K, Terauchi Y, Ueki K, Kadowaki T. Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. Cell Metab. 2008 Jul;8(1):49-64.

15. Previs SF, Withers DJ, Ren JM, White MF, Shulman GI. Contrasting effects of IRS-1 vs IRS-2 gene disruption on carbohydrate and lipid metabolism in vivo. J Biol Chem. 2000 Sep 19;275(50):38990-4.

16. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet. 1999 Sep;23(1):32-40.

17. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006 Dec 14;444(7121):860-7.

18. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL, Shulman GI. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci USA 2007 Jul 31;104(31):12587-94.

19. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, Vottero A, Kanabar D, Charlton-Menys V, Durrington P, Soos MA, Carpenter TA, Lomas DJ, Cochran EK, Gorden P, O'Rahilly S, Savage DB. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. J Clin Invest. 2009 Feb;119(2):315-22.

20. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004 Jul;88(4):787-835, ix.

21. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev. 1995 Jul;75(3):473-86.

22. Kitabchi AE, Nyenwe EA. Hyperglycemic crises in diabetes mellitus: diabetic ketoacidosis and hyperglycemic hyperosmolar state. Endocrinol Metab Clin North Am 2006 Dec;35(4):725-51, viii.

23. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005 Jun; 54 (6):1615-25.