DOES BODY MASS INDEX MEDIATE THE ASSOCIATION BETWEEN MEAT INTAKE AND INSULIN SENSITIVITY?

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Abstract

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The purpose of this study was to examine the possible inverse relationship between total meat intake or processed meat intake on insulin sensitivity as mediated by body mass index (BMI). The Quantitative Insulin Sensitivity Check Index (QUICKI) was used to calculate participants' insulin sensitivity. Cross-sectional data were used from Visit 1 of the Atherosclerosis Risk In Communities cohort. Mediation analysis was conducted using the PROCESS macro for SPSS. The mean BMI was 27.3 kg/m2, total meat intake was 1.76 servings per day, processed meat intake was 0.44 servings per day, and the mean QUICKI score was 0.34. As hypothesized, total meat intake was significantly associated with lower insulin sensitivity. The significant inverse total association model was -0.0044 (95% CI: -0.0054, -0.0034) with a significant inverse BMI association [-0.0029 (95% CI: -0.0033, -0.0024)] which indirectly accounted for 66% of the association between total meat intake and QUICKI. The processed meat intake association was partially mediated by BMI. Ultimately, these findings suggest that the majority of the association of meat intake with insulin sensitivity is contributed by its association with BMI.

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Foreword

Chapter 2 of this thesis will be submitted to *Nutrients*, an international open access peer-reviewed scientific journal published by Multidisciplinary Digital Publishing Institute (MDPI); it has been formatted according to the style guide for that journal.

Chapter 1: Literature Review

Diet and lifestyle choices have an enormous impact on our health and well-being. Cardiovascular diseases [1], diabetes [2], some cancers [3], non-alcoholic fatty liver disease [4], and obesity [5,6] are all influenced by diet and lifestyle choices. In particular, prevention and treatment for type 2 diabetes is directly influenced by diet and lifestyle [7]. The rapidly growing rate of type 2 diabetic incidence is one of the most acute health problems facing the world today. Between 2010 and 2030, adult type 2 diabetes incidence is predicted to increase by 69% in developing countries and 20% in developed countries [8]. The global prevalence of diabetes among adults aged 20 to 79 years will increase to 7.7% by 2030, affecting 439 million people worldwide [8].

Overall meat consumption, along with type 2 diabetic incidence and insulin resistance, is increasing worldwide. Currently in the USA, poultry is preferred over red meat; however, red meat makes up 58% of meat consumed and 22% of all meat is processed. The average US intake of total meat is 128 g/day [9]. Positive correlations between meat and insulin resistance are described in several epidemiological studies.

Insulin resistance is a well-known, key characteristic of type 2 diabetes [10]. Insulin resistance occurs when cells do not respond properly to insulin, resulting in chronic hyperglycemia. Insulin resistance is a component of metabolic syndrome [11], and is found in other chronic diseases [12]. Insulin resistance is associated with low-grade tissue-specific inflammation; the inflammatory response is thought to be activated by various oxidative stress and pro-inflammatory mediators. Over a period of time, chronic exposure to oxidative stress and pro-

inflammatory mediators can influence pancreatic ß-cell dysfunction and thus contributes to insulin resistance [13].

Fretts et al. found higher insulin concentrations and higher fasting glucose results related to red meat intake, regardless of genetic risk, in a population of 50,345 Caucasians [14]. When BMI was adjusted for, the relationship between red meats and fasting blood glucose and fasting insulin concentrations were attenuated. Fretts et al. concluded meat intake is linked with higher glucose and insulin levels. Ley et al. used diabetes-free, female participants from the Nurse's Health Study to assess the associations between total, unprocessed, and processed red meat consumption and inflammatory and glucose metabolic markers [15]. Red meat consumption was positively associated with the selected inflammatory and glucose metabolic markers, with the exception of adiponectin, which was negatively associated. After further adjustment for medical and lifestyle factors, the association between meat consumption and adiponectin became attenuated. When body mass index (BMI) was included in the model, the remaining markers became attenuated and no longer statistically significant; BMI also attenuated the association between CRP, HbA1c, and fasting insulin. The researchers concluded red meat consumption was associated with unfavorable inflammatory and glucose metabolic markers.

Not all epidemiologic studies found the same attenuation after adjustment for BMI. Lee et al., using the multiethnic Insulin Resistance Atherosclerosis Study (IRAS) cohort, found fish consumption was not associated with fasting insulin, direct measures of insulin sensitivity or beta cell dysfunction in African Americans or Hispanics [16]. However, Caucasians who consumed ≥2 portions of fish per week were more likely to have a lower insulin sensitivity-adjusted acute insulin response, higher fasting blood glucose, and increased beta cell dysfunction

than those who did not. These associations remained even after adjustment for potential demographic, socioeconomic, clinical, lifestyle, and diet confounders. It is of note that the two previous studies focused on red meat and processed red meats and their relationship to insulin sensitivity, while Lee et al. focused on fish consumption and subsequent relationship to insulin sensitivity.

One study did not find a positive relationship between insulin resistance and red meat consumption. Turner et al. conducted a randomized crossover human trial looking at insulin sensitivity in overweight and obese individuals after a high-red meat diet, a high-dairy diet, and a control diet [17]. The high lean meat diet did not have a different effect on insulin and glucose responses when compared to the control diet. However, they found the high-dairy diet did reduce insulin sensitivity by 14.7% in women when compared to the high-red meat diet. The researchers concluded it was high dairy intake rather than high lean meat intake that contributed more to reduced insulin sensitivity.

Two randomized control trials found fish protein improved insulin sensitivity. Ouellet et al. found a cod protein diet improves insulin sensitivity in insulin-resistant men and women when compared to a diet consisting of other animal proteins (beef, pork, veal, and milk) [18]. They attributed the beneficial effects of cod protein (which is high in arginine and low in branch-chain amino acids) on insulin sensitivity to cod's amino acid composition acting on the insulin signaling pathway. However, the researchers did note it was possible ω-3 polyunsaturated fatty acids (PUFA)s also contributed to cod's beneficial findings as the ω-3 PUFA content found in plasma phospholipids was higher in those who consumed them in the form of cod, rather than when supplemental cod liver oil was added to the mammalian protein diet.

Navas-Carretero et al. conducted a randomized crossover dietary intervention to determine if an oily fish diet improved insulin sensitivity over a red meat diet in iron-deficient young Spanish women [19]. The researchers found BMI and blood pressure remained constant; total cholesterol and LDL-cholesterol decreased equally. HDL-cholesterol improved during both treatments; however, the oily fish diet improved HDL-cholesterol more than the red meat diet. Oxidation and inflammation markers were not statistically different between the two treatments. The oily fish diet significantly improved insulin sensitivity when compared to the red meat diet; this finding was attributed to the EPA and DHA content of oily fish.

This association observed between meat intake and insulin resistance is possibly related to common pro-inflammatory compounds found in meat [20]. A meta-analysis looking at unprocessed and processed red meats and risk of coronary heart disease and diabetes revealed both processed and red meats are associated with a higher risk for developing diabetes. The researchers found a higher risk per gram daily intake for processed meats compared to unprocessed red meat [21]. Potential underlying mechanisms discussed were the heme-iron content of meats, preservatives (like sodium, nitrites, and nitrates), and different meat preparation methods. The researchers concluded saturated fat was an unlikely underlying mechanism due to the similar average saturated fat content between processed and unprocessed red meat. Dietary cholesterol was cited as having the potential to contribute to the development of type 2 diabetes and coronary heart disease, although it did not account for the high diabetes risk seen with processed meats. Further discussion in this literature review will examine heme-iron, preservatives, dietary L-carnitine, and advanced glycation end-products (AGEs) produced as a result of cooking and their relevance to insulin resistance and diabetes.

Jehn et al. used the 1987-1989 Visit 1 Atherosclerosis Risk in Communities (ARIC) cohort, to examine the relationship between plasma ferritin levels and incident diabetes; this is the same cohort used in the present study [22]. Jehn et al. found elevated body iron stores were associated with increased risk of type 2 diabetes. The researchers posed two theories for this relationship: elevated iron stores, as evidenced by elevated plasma ferritin, may cause metabolic abnormalities resulting in diabetes or elevated serum ferritin may be another metabolic abnormality indirectly related to diabetes rather than a causal factor. There were no mentions of any possible dietary factors in this study.

Hansen, Moen, and Mandrup-Poulsen reviewed iron and diabetes pathophysiology. They concluded iron is essential for normal ß-cell function and normal glucose regulation, but excess iron is toxic and can contribute to the pathophysiology of both type 1 diabetes and type 2 diabetes [23]. The primary toxic effect of iron overload is not clear, but two possible mechanisms were provided: pro-inflammatory reactive oxygen species (ROS) generated from excess iron resulting in ß-cell dysfunction and excess iron build up in ß-cell mitochondria. Excess iron accumulation in the mitochondrial dysfunction caused by iron build up can inhibit ATP generation and insulin secretion. The review article goes on to mention the protective mechanism of reducing iron in ß-cell is not well understood. There is support from epidemiologic studies, but limited human randomized control trials addressing iron reduction and improvement in diabetes; evidence from animal studies indicate decreased iron consumption improves insulin secretion and supports normal glucose regulation [23]. However, care must be taken when recommending decreased iron consumption in populations vulnerable to iron deficiency.

There are few studies concerning preservatives and their relation to diabetes in general and even fewer human studies available. Sodium has a well-documented relationship with cardiovascular diseases, but there is limited research examining its direct effect on incident diabetes. Other preservatives, like nitrites in the form of sodium nitrite, are commonly found in processed and cured meats [24]. Nitrites have been called in question due to their ability to form carcinogenic nitrosamines under certain conditions. However, Sindelar and Milkowski reviewed controversies surrounding nitrates and nitrites in the human diet and concluded there is growing evidence supporting nitrites and nitrates as generally safe for human consumption [25]. They reported that after nitrosamine formation in cured meats was identified in 1971, regulations were put into place to reduce the potential for nitrosamine formation in bacon. The researches later go on to report, "Today the regulatory controls, and more stringent plant production practices have essentially eliminated all regulatory nitrosamine concerns in meats and poultry products." making nitrosamines an unlikely concern in influencing insulin resistance.

Dietary L-carnitine is abundantly found in red meat and is linked to atherosclerosis [26]. Koeth et al. found L-carnitine, through a human-gut microbiota dependent mechanism, produces trimethylamine N-oxide (TMAO), a pro-atherosclerotic compound, and ultimately decreased cardiovascular function. However, a meta-analysis of five randomized control studies examined L-carnitine supplementation for treatment of insulin resistance as evaluated by HOMA-IR [26]. Xu et al. found L-carnitine supplementation improved insulin sensitivity. However, the researchers concluded these findings needed more validation from further randomized control trials and had several criticisms concerning the reviewed studies' designs [27].

Advanced glycation end products are diverse group of highly oxidant glycotoxins formed through a nonenzymatic reaction between reducing sugars and free amino acids, this

reaction is also known as the Maillard or browning reaction in culinary terms [28]. Toxic effects are observed when high levels of AGEs accumulate in tissues and circulation [Urribari]; serum levels of AGEs correlate with dietary AGEs intake [29]. Poulsen et al. reviewed dietary AGEs and subsequent health effects; several epidemiological studies found serum concentration of AGEs were positively associated with both type 1 diabetes and type 2 diabetes; these associations were related to macrovascular and microvascular complications, endothelial dysfunction, and increased inflammation. After reviews of animal trials, human trials, and epidemiological studies, the researchers concluded diets with fewer AGEs may have a substantial impact on prevention of diabetes and diabetes-related complications [29].

Despite many epidemiological studies finding positive correlations between meat and insulin resistance in several different cohort, adding BMI into the model often attenuates the association; randomized control trials also seen mixed results. Obesity is a well-known risk factor in the development of diabetes and other metabolic abnormalities and is also positively correlated with red and processed meat intake [30]. Ceriello and Motz reviewed the pathogenic mechanism underlying insulin resistance and chronic disease. The reviewers proposed oxidative stress as a pathogenic mechanism for insulin resistance and ß-cells and endothelial dysfunction [31]. A proposed mechanism for obesity-induced insulin resistance is that cells, in times of caloric excess, will try to prevent or reduce reactive oxygen species (ROS) formation in order to protect against reactive oxygen species (ROS) related damage [31]. This occurs by inhibiting insulin-stimulated nutrient uptake and preventing energetic substrates entering the mitochondria; in this context insulin resistance can be understood as a corrective mechanism against further oxidative damage. ß-cells are vulnerable to ROS-damage because they are low in antioxidant enzymes [31].

Low-level chronic inflammation is also typically present in obesity and may influence development of insulin resistance and endothelial dysfunction [32, 33]. This low level chronic inflammation is the consequence of adipocyte dysfunction thought to be caused by an excess of adipose tissue [34]; the adipocyte will secrete pro-inflammatory compounds including, but not limited to, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, interleukin (IL)-1, IL-6, and IL-8, all are linked to insulin resistance [34]. Obese individuals also tend to have lower intakes of antioxidant and phytochemical rich foods, this trend is documented in the United States, European countries, New Zealand, Canada, South American countries, and Asian countries [35]. Fruits and vegetables contain antioxidants and are associated with reduced risk of chronic diseases and obesity [36].

Unlike fruits and vegetables, meat consumption is associated with obesity in observational studies [37]. Vergnaud et al. found meat consumption was positively associated with weight in very large cohort of women from 10 different European countries [37]. This association was also found by Wang and Beydoun using the National Health and Nutrition Examination Survey, however they found a stronger positive relationship with waist circumference than with body mass index, although both were significant [36]. Diets high in meat intake are also correlated with poor quality food choices. Fogelholm et al. found diets high in meat were inversely associated with fruit, whole grain, and nut consumption in a nationally representative Finnish cohort [38]. A meta-analysis of 98 global studies examined health risk factors associated with meat, fruit, and vegetable consumption. The researchers found a 100 g per day increase in red meat consumption was associated with increased BMI; fruit and vegetable consumption was positively associated with high education, high physical activity, and non-smok-

ing status. However, strength of association varied between geographic regions [39]. This suggests that meat consumption in conjunction with other lifestyle factors influences risk of obesity.

The above review details the discrepancies between epidemiological and randomized control studies. Previous epidemiological studies have found a positive relationship between meat intake and increased measures of insulin resistance; however, those relationships have been largely attenuated when body mass index is included in their models [14, 15]. Beyond simply commenting on BMI's tendency to attenuate any relationship found between meat intake and insulin resistance, few epidemiologic studies have further investigated the interrelationship between BMI, meat intake, and insulin resistance. Based on these considerations, BMI may constitute a mechanism that indirectly influences meat intake and insulin resistance. Mediation analysis is a statistical tool allowing for further exploration into BMI as a mediating variable. Andrew Hayes, a well-known mediation-analysis expert writes "a simple mediation model is any causal system in which at least one causal antecedent X variable is proposed as influencing an outcome Y throughout a single intervening variable M. In such a model there are two distinct pathways by which a specific X variable is proposed as influencing Y" [40]. The two pathways Hayes is speaking of are the direct and indirect effects; the direct effect indicates the association of X on Y, the indirect effect is how X influences M and subsequently how M influences Y. This analysis provides another way to understand BMI's relationship to meat intake and insulin resistance in an epidemiological study.

Mediation analysis has been used previously to understand the relationship between body mass index (BMI), health outcomes and potential causal factors [41, 42]. However, no

current research has examined the relationship between meat intake, diabetes risk, and BMI using mediation analysis before this study.

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Chapter 2: Article

Does Body Mass Index Mediate The Association Between Meat Intake and Insulin Sensitivity?

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Abstract

The purpose of this study was to examine the possible inverse relationship between total meat intake or processed meat intake on insulin sensitivity as mediated by body mass index (BMI). The Quantitative Insulin Sensitivity Check Index (QUICKI) was used to calculate participants' insulin sensitivity. Cross-sectional data were used from Visit 1 of the Atherosclerosis Risk In Communities cohort. Mediation analysis was conducted using the PROCESS macro for SPSS. The mean BMI was 27.3 kg/m2, total meat intake was 1.76 servings per day, processed meat intake was 0.44 servings per day, and the mean QUICKI score was 0.34. As hypothesized, total meat intake was significantly associated with lower insulin sensitivity. The significant inverse total association model was -0.0044 (95% CI: -0.0054, -0.0034) with a significant inverse BMI association [-0.0029 (95% CI: -0.0033, -0.0024)] which indirectly accounted for 66% of the association between total meat intake and QUICKI. The processed meat intake association was partially mediated by BMI. Ultimately, these findings suggest that the majority of the association of meat intake with insulin sensitivity is contributed by its association with BMI.

Keywords: body mass index; insulin sensitivity; meat intake; mediation

1. Introduction

Insulin resistance is a pathological condition underlying type 2 diabetes; it is also associated with other chronic conditions like hypertension, dyslipidemia, obesity, and non-alcoholic fatty liver disease [1]. Prevalence of these chronic conditions is expected to rise over the next few decades. For type 2 diabetes alone, incidence is predicted to increase by 69% for adults in developing countries and by 20% in developed countries between 2010 and 2030 [2]. Global prevalence of diabetes among adults aged 20 to 79 years will increase by 7.7% by 2030, affecting 439 million people worldwide [2]. Along with type 2 diabetic incidence, overall meat consumption is increasing worldwide. Currently in the USA, red meat makes up 58% of meat consumed and 22% of all meat is processed; however there is a growing preference for poultry over red meat [3]. Regular meat consumption has been linked to several chronic conditions like hypertension, hyperglycemia, and heart disease. This association is possibly related to common pro-inflammatory compounds found in cooked meat, such as saturated and trans fats, sodium, nitrites, nitrosamines, heme-iron, and advanced glycation end products (AGEs) [4]. Previous epidemiological studies have found a positive relationship between meat intake and increased measures of insulin resistance; however, those relationships have largely been attenuated when body mass index (BMI) is included in their models [5,6]. Low-level chronic inflammation is typically present in obesity and may influence development of insulin resistance and endothelial dysfunction [7]. Beyond simply commenting on BMI's tendency to attenuate any relationship found between meat intake and insulin resistance, few epidemiologic studies have further investigated the interrelationship between BMI, meat intake, and insulin resistance. The present study was designed to investigate BMI as a mediating variable between meat intake and measures of insulin resistance.

2. Materials and Methods

2.1 Atherosclerosis Risk in Communities Cohort Description and Participant Selection

This manuscript was prepared using Atherosclerosis Risk in Communities (ARIC) Research Materials obtained from the National Heart Lung Blood Institute (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center and does not necessarily reflect the opinions or views of the ARIC research groups or the NHLBI. The Institutional Review Board of Appalachian State University approved the acquisition and use of this dataset. The ARIC Study is a prospective epidemiological study sponsored by the National Heart, Lung, and Blood Institute. It was originally designed to investigate the causes of cardiovascular disease in a biracial population [8]. A total of 15,792 randomly selected participants from four field centers in (Washington County, MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN) completed extensive medical, social, dietary, and demographic examinations. Participants were re-examined four separate times, three years apart, beginning in 1987 through 1998 [9]. Data from the first examination, which was undertaken between 1987 and 1989, were used for cross-sectional analysis. Participants were excluded if they had a diagnosis of diabetes or a fasting blood glucose measurement > 126mg/dL. Outliers were defined as ≥ 4 servings/day of poultry, ≥ 4 servings/day of red meat, ≥ 3 servings/day of processed meat, ≥2 servings/day of fish, and ≥ 8 servings/day of total meat intake. Final sample size = 12,908 participants. See Figure 1 for flow chart describing participant selection in more detail. Information was gathered from the derived, diet questionnaire, and nutritional datasets.

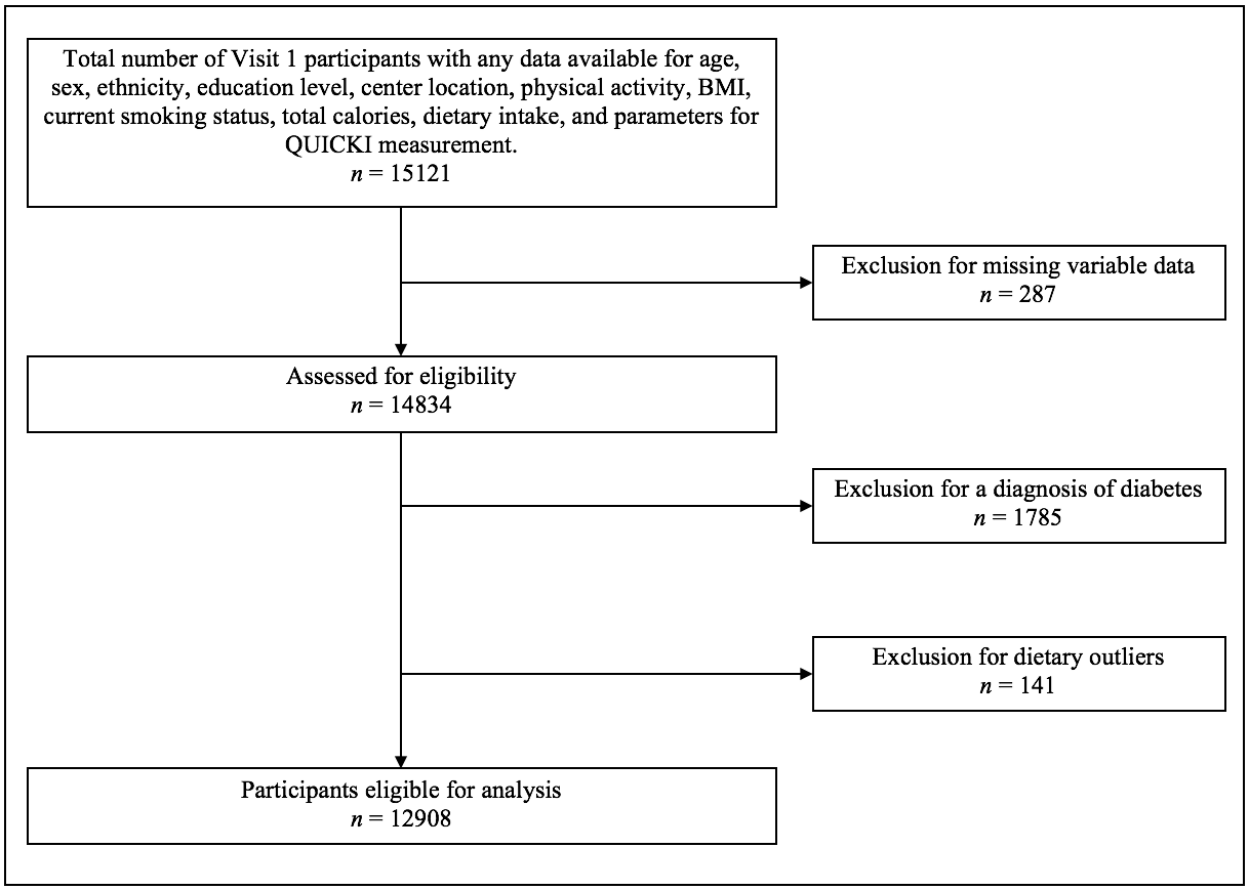

Figure 1. Flow diagram of participant selection

2.2 Description of QUICKI Measurement

The Quantitative Insulin Sensitivity Check Index (QUICKI) was developed to quickly and accurately measure insulin sensitivity from fasting plasma insulin levels (microunits per mL) and fasting blood glucose levels (milligrams per dL) [10]. The QUICKI equation is similar to the Homeostasis model assessment of Insulin Resistance (HOMA-IR) equation, with the exception of the reciprocal of the log transformation used in QUICKI. Lower scores indicate reduced insulin sensitivity; a score of <0.36 has been observed in patients with metabolic syndrome [11]. The equation is: 1 / (log(fasting insulin μU/mL) + log(fasting glucose mg/dL)).

2.3 Dietary Analysis

The food frequency questionnaire (FFQ) assessed meat consumption by asking: "In the past year, how often on average did you consume…" Participants could then answer from the following categories: >6 per day, 4-6 per day, 2-3 per day, 1 per day, 5-6 per week, 2-4 per week, 1 per week, 1-3 per month, or almost never. Servings per day were calculated to be 8, 5, 2.5, 1, 0.786, 0.429, 0.143, 0.067, or 0, respectively. Red meat included beef, pork, and lamb; consumption of red meat was considered to be one each per serving of hamburgers, mixed red meat dishes (i.e., stew, lasagna, sandwiches), and red meat as an entrée (i.e., roast, steak, and/or ham as a main dish). Poultry included chicken and turkey; consumption of poultry was considered to be chicken or turkey with and without the skin. Fish included shellfish and fish; consumption of fish was considered to be 3-5 oz. per serving of dark meat fish (i.e., salmon, mackerel, swordfish, sardines, or bluefish), 3-5 oz. per serving of other fish (i.e., cod, perch, catfish), 3-4 oz. per serving of canned tuna, and shellfish as an entrée (i.e., shrimp, lobster, or scallops as a main dish). Processed meat included sausage, salami, bologna, bacon and hot dogs; consumption of processed meat was considered to be one piece or slice per serving of sausage, salami, and bologna, two slices per serving of bacon, and one each per serving of hot dogs. Total meat intake was calculated as the sum of red meat, poultry, fish, and processed meat intake. Specific weight serving sizes were not mentioned in questionnaire description for red meat, poultry, shellfish, and processed meats. Fruit included one each per serving of fresh apples, pears, oranges, and bananas; a small glass of orange or grapefruit juice, one each fresh or ½ cup canned or dried per serving peaches, apricots, plums, and other fruits (including fruit cocktail). Vegetable intake was ½ cup per serving of string or green beans, broccoli, cauliflower, brussel sprouts, peas or lima beans, sweet potatoes, dark yellow winter squash (i.e.,

acorn, butternut), beans or lentils, spinach, collards, or other greens (excluding lettuce); one each whole or ½ cup cooked per serving of carrots, one each per serving or ½ cup per serving of corn; and one each per serving of tomatoes or 4 oz. of tomato juice. Fruit and vegetable intake was calculated as the sum of all fruits and vegetables listed above.

2.4 Other variables

Physical activity was calculated by taking the sum of the three listed physical activity indices – work index (definition two), sport index (definition two), and leisure time index (definition one). A higher physical activity score indicates a higher level of physical activity, participants could score 1-15. Using variable definition two, education level was divided into three categories: 1 – Basic education (no education or <12th grade education), 2 – Intermediate education (high school diploma, GED, or up to three years vocational school), 3 – Advanced education (minimum 1 year of college to graduate or professional school). Current smoking status was determined by the questionnaire asking, "Have you ever smoked cigarettes?" and "Do you now smoke cigarettes?" Current smokers were those who answered "yes" for both questions.

2.5 Statistical Analysis

Means and standard deviations were used to describe key variables (age, BMI, physical activity, total calories, fruit and vegetable intake, red meat intake, poultry intake, fish intake, processed meat intake, total meat intake, and QUICKI). Percentages were used to describe ethnicity, sex, smoking status, and education level. Using linear regression, univariate, demographic, and two multivariate models (one with BMI and one without) were created using QUICKI as the dependent variable and total meat intake or processed meat intake as independ-

ent variables of interest. Mediation analysis was used to further analyze the relationship between total or processed meat intake and QUICKI as mediated through BMI. This analysis was conducted using ordinary least squares path analysis with the PROCCESS macro for SPSS v. 24 [12]. The first mediation model was generated using total meat as the independent variable, BMI as the mediating variable, QUICKI as the dependent variable and the same covariates as the first multivariate linear regression model using total meat intake. The second mediation model was generated using processed meat as the independent variable, QUICKI as the dependent variable and the same covariates as the first multivariate model using processed meat intake. After finding significant results, sensitivity analysis were conducted. Three sensitivity analyses were performed on multivariate models 1 and 2 for both total meat intake and processed meat intake. The first sensitivity analysis included participants who had diabetes but still excluded dietary outliers. The second sensitivity analysis excluded participants with diabetes and included dietary outliers. The third sensitivity analysis included both participants with diabetes and dietary outliers.

3. Results

3.1 Descriptive Statistics

Table 1 shows the characteristics of the study population. The majority of participants were middle-aged white females; participants were also typically overweight with a QUICKI score indicating insulin resistance (<0.36). On average, participants were moderately active, with the mean falling just below half on the physical activity score. Total meat consumption was approximately 1 ¾ servings per day. Thirty-seven percent of participants had at least some college education.

Table 1. Characteristics of study sample.

Variables	Mean (n = 12908)	Standard Deviation
Age (years)	54.0	5.7
BMI (kg/m^2)	27.3	5.0
QUICKI	0.34	0.39
Physical Activity Score	7.0	1.4
Total Calorie Intake (kcal)	1623	648
Blood Glucose (mg/dL)	98.64	9.36
Red Meat Intake (servings/d)	0.64	0.47
Poultry Intake (servings/d)	0.35	0.30
Fish Intake (servings/d)	0.31	0.29
Processed Meat Intake (servings/d)	0.44	0.46
Total Meat Intake (servings/d)	1.76	0.89
	Percentage	
Female Sex	55%	
Black Ethnicity	23%	
Current Smoker	26%	
Advanced Education	37%	

BMI, body mass index; QUICKI, quantitative insulin sensitivity check

3.2 Linear Regression Models

Table 2. Relation between total meat intake and insulin sensitivity (QUICKI)

Models	B	CI of B	p
Univariate	-.0043	-.0051, -.0036	<.001
Demographic	-.0032	-.0039, -.0024	<.001
Multivariate 1	-.0044	-.0054, -.0034	<.001
Multivariate 2	-.0015	-.0024, -.0007	<.001

CI, confidence interval; Betas and p-values for trend are the result of linear regression of total meat intake (servings/d) on insulin sensitivity (QUICKI). The univariate model predicted total meat intake on insulin sensitivity with no covariates. The demographic model controlled for age, black ethnicity, and female sex. Multivariate 1 also controlled for education level, smoking status, study center location, physical activity level, fruit and vegetable intake, and total calorie intake. Multivariate 2 included Multivariate 1 and BMI.

Table 3. Relation between processed meat intake and insulin sensitivity (QUICKI)

Models	B	CI of B	p
Univariate	-.0081	-.0096, -.0067	<.001
Demographic	-.0048	-.0063, -.0033	<.001
Multivariate 1	-.0047	-.0064, -.0030	<.001
Multivariate 2	-.0022	-.0037, -.0007	.003

CI, confidence interval; Betas and p-values for trend are the result of linear regression of processed meat intake (servings/d) on insulin sensitivity (QUICKI). The univariate model predicted processed meat intake on insulin sensitivity with no covariates. The demographic model controlled for age, black ethnicity, and female sex. Multivariate 1 also controlled for education level, smoking status, study center location, physical activity level, fruit and vegetable intake, and total calorie intake. Multivariate 2 included Multivariate 1 and BMI.

3.3 Mediation Analysis

Mediation analysis is described in Figure 2 and 3 for total meat and processed meat respectively. The relationship between total meat intake and QUICKI was mediated by BMI by 66%. This number was calculated by dividing the indirect effect (-0.0029) over the total effect (-0.0044) using information in Figure 2. The relationship between processed meat intake and QUICKI was partially mediated by BMI by 53%. This number was also calculated using information in Figure 3. Sensitivity analyses were robust. Results remained significant regardless of inclusion/exclusion criteria.

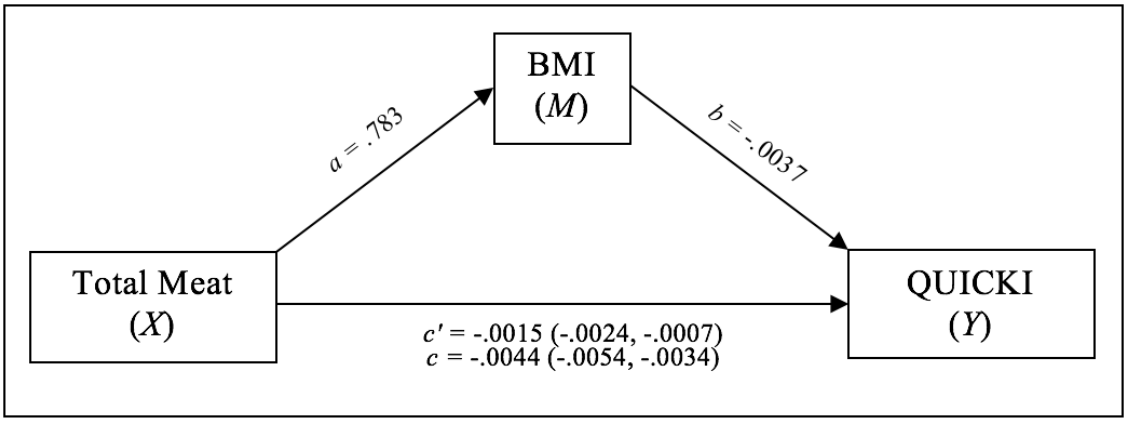

Figure 2. Mediation analysis of total meat intake through BMI on insulin sensitivity (QUICKI). Statistical diagram for processed meat mediation model. 95% CI given in parenthesis. a = direct effect of X on M; b = direct effect of M on Y; c' = direct effect of X on Y; c = total effect (ab + c'). The indirect effect is calculated as the product of a and b; ab = -0.0029 (-0.0033, -0.0024).

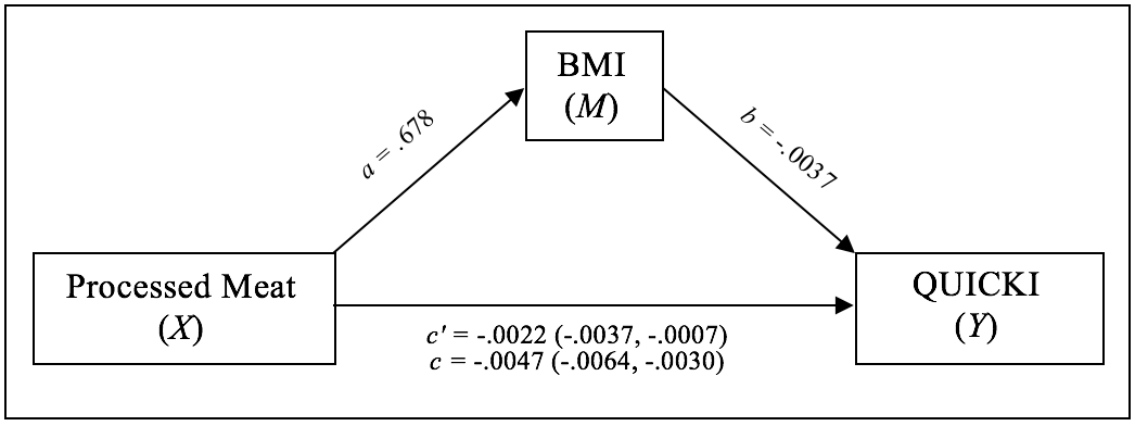

Figure 3. Mediation analysis of processed meat intake through BMI on insulin sensitivity (QUICKI). Statistical diagram for processed meat mediation model. 95% CI given in parenthesis. a = direct effect of X on M; b = direct effect of M on Y; c' = direct effect of X on Y; c = total effect (ab + c'). The indirect effect is calculated as the product of a and b; ab = -.0025 (-.0033, -.0016).

4. Discussion

4.1 Summarization of results

The results from the total meat analysis suggested that total meat intake was significantly associated with reduced insulin sensitivity. It was hypothesized that these effects could be mediated by BMI, considering sizable BMI effect to the B values multivariate models 1 and 2 for total meat intake. Mediation analysis revealed that BMI indirectly accounted for 66% of the inverse association seen between total meat intake and insulin sensitivity. Processed meat intake was similarly associated with reduced insulin sensitivity and model 2 was similarly affected by BMI. Mediation analysis for processed meat-BMI-insulin sensitivity showed that BMI partially mediated 53% of the relationship, indicating that some, but not all of the relationship between the processed meat intake and QUICKI was mediated by BMI. However, this relationship cannot be considered to be conclusive due to overlapping confidence intervals between the direct (-0.0022, 95% CI: -0.0037, -0.0007) and indirect (-0.0025, 95% CI: -0.0033, -0.0016) effects. In this circumstance overlapping CIs indicate that it is not possible to tell where or if the mediation is happening as the direct and indirect effects appear to be equally strong.

4.2 Possible mechanistic explanations

The significant association seen between both types of meat and insulin sensitivity may be related to compounds often found in all meats and particularly in processed meats. Suspected compounds reported by others include iron, L-carnitine, added nitrites in processed meats, and advanced glycation end products (AGEs) [13]. While critical for normal metabolism, excess cellular iron is pro-inflammatory and may cause metabolic abnormalities that result in ß-cell dysfunction. Elevated serum ferritin, a measure of iron stores, has been linked

with incident diabetes [14]. However, whether excess serum iron has direct or indirect causal relationship with diabetes remains unclear [14, 15]. L-carnitine was associated with trimethylamine-N-oxide (TMAO), a pro-atherosclerotic compound [16]; however, a meta-analysis examining L-carnitine and insulin sensitivity tenuously found that L-carnitine may actually improve insulin sensitivity [17]. Despite periodic controversy and inconsistent scientific evidence suggesting otherwise, nitrites are considered safe for human consumption [18]. Added nitrites have the potential to form carcinogenic nitrosamines under specific conditions, like frying bacon [18]. However, regulations put in place have significantly reduced the potential for nitrosamine formation in processed meats [18]. AGEs are a diverse group of highly oxidant glycotoxins formed as a product of the Maillard or browning reaction seen between meats and certain cooking techniques like grilling and frying. Dietary AGEs have been positively associated with macrovascular and microvascular complications, endothelial dysfunction, and increased inflammation. A review looking at AGEs and disease risk concluded that a diet low in AGEs may be beneficial in reducing risk for developing diabetes and preventing diabetes-related complications [19].

The indirect effect of BMI may be easier to pinpoint. Obesity is a well-known risk factor in the development of diabetes and other metabolic abnormalities. Chronic oxidative stress is a feature of obesity and may contribute to the development of chronic diseases through production of reactive oxygen species (ROS). One of the ways ROS formation can be generated is through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which is a cellular response protein. In the presence of oxidative stress, p65, a subunit of the NF-kB pathway, is activated, leading to increased cytokine production and gene encryption of

monocyte chemoprotein-1. This leads to increased production of ROS, and ultimately increased levels of inflammation. [20]. In addition to ROS related oxidative stress, adipose tissue is a recognized endocrine organ. Excess adipose tissue and adipocyte dysfunction, commonly found in overweight and obese individuals, can result in dysregulation of the adipose tissue-derived secretory factors known as adipokines [21]. In a state of obesity, the adipocyte increases the secretion of pro-inflammatory compounds such as tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, interleukin (IL)-1, IL-6, and IL-8; these compounds have also been linked to insulin resistance [21].

A proposed mechanism for obesity induced insulin resistance is that the peripheral cells, in times of caloric excess, in order to protect against ROS-related damage will try to prevent or reduce ROS formation [22]. This occurs by inhibiting insulin-stimulated nutrient uptake and preventing energetic substrates entering the mitochondria; in this context insulin resistance can be understood as a corrective mechanism against further oxidative damage. ß-cells are vulnerable to ROS-damage because they are low in antioxidant enzymes [22]. Obese individuals tend to have lower intakes of antioxidant and phytochemical rich foods, this trend has been documented in the United States, European countries, New Zealand, Canada, South American countries, and Asian countries [23]. Fruits and vegetables contain many beneficial nutrients, including the anti-oxidant vitamins C and E, potassium as well as, flavonoids, polyphenols, and fiber. Fruits and vegetable intake has been associated with reduced risk of obesity and type 2 diabetes [24].

Meat consumption has been associated with obesity in observational studies [25]. Vergnaud et al. found that meat consumption was positively associated with weight in very large cohort of women from 10 different European countries [25]. This association was also

found by Wang and Beydoun using the National Health and Nutrition Examination Survey, however they found a stronger positive relationship with waist circumference than with BMI, although both were significant [26]. Diets high in meat intake have also been correlated with poor quality food choices. Fogelholm et al. found that diets high in meat were inversely associated with fruit, whole grain, and nut consumption in a nationally representative Finnish cohort [27]. A meta-analysis of 98 global studies examined health risk factors associated with meat, fruit, and vegetable consumption. The researchers found that a 100 g per day increase in red meat consumption was associated with increased BMI; fruit and vegetable consumption was positively associated with high education, high physical activity, and non-smoking status. However, strength of association varied between geographic regions [28]. Meat intake has been associated with diabetes risk both as a part of a larger dietary pattern, but also as an independent risk factor. Fung et al. assessed major dietary patterns and risk of type 2 diabetes in women. They found that "Western-style" diets, which are high in red and processed meats, refined grains, sugar, and low fruit and vegetable intake, were associated with an increased risk for diabetes; in addition to these findings, red and processed meats were also indirectly associated with diabetes risk [29].

4.3 Strengths and limitations of this study

The main strength of our study was the further exploration of the association between meat intake and insulin resistance with the use of mediation analysis. With previous epidemiologic research [5,6], statistical analyses using multiple linear regression concluded with BMI as an attenuating factor. This study brought more insight into BMI's relationship with the two main variables of interest. Other strengths include a large sample size derived from four U.S. geographical regions, representation from both sexes and Whites and African Americans, and

a homogenous population of late-middle aged adults. This is also a fairly novel study; to the best of the researcher's knowledge, this is the first study to examine the relationship between meat intake and insulin sensitivity as mediated by BMI.

Our study design had several limitations: The data were cross-sectional; therefore, no causal inferences can be assumed. However, our findings would support the causal model of total meat consumption leading to a higher BMI, thus in turn leading to decreased insulin sensitivity. Although we adjusted for several potential confounding variables, potential bias from unmeasured or poorly measured factors may be present; there is no way to validate the accuracy of the dietary assessment due to possible self-reporting inaccuracies and questionnaire limitations. Finally, the results may not be generalizable to other populations.

4.4 Conclusions and public health limitations

In conclusion, the results of this study help explain the relationship between meat intake, BMI, and markers of insulin sensitivity in a 45-65 year old, predominantly white American population. Both total meat and processed meat intakes had inverse relationships with measures of insulin sensitivity. The impact of total meat intake on insulin sensitivity was largely mediated by BMI. Processed meat intake did have an effect on insulin sensitivity; it was partially mediated by BMI. The indirect and direct effects appeared to be equally strong, therefore, no conclusions can be made because it is not possible to determine where or if mediation occurred. Ultimately, this study supports the current dietary guidelines that advise reducing red meat consumption. However, if meat is to be included in the diet, choosing lean, unprocessed cuts of meat and eating in moderation is recommended; as well as, using acidic marinades and avoiding broiling, frying, and grilling [30].

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Author Contributions: A.C, M.R. and C.E. conceived and designed study; A.C. analyzed data; M.R. contributed to analysis interpretation; A.C. wrote the manuscript; M.R. edited the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Vita

Abigale Rae Clapham was born in Bangor, Maine but calls Greenbrier County, West Virginia home. She is the daughter of William and Sarah Clapham. She graduated from Greenbrier East High School in West Virginia in 2011. That fall, she entered Shepherd University to study Biology. She later transferred to West Virginia University, starting January of 2013, initially seeking a degree in Animal Nutritional Sciences; the following semester she changed majors to study human nutrition. In May 2016, Ms. Clapham was awarded a Bachelor of Science in Human Nutrition and Foods. In the fall of 2016, she started a dual dietetic internship and graduate program at Appalachian State University. Ms. Clapham completed her dietetic internship and was awarded a Master of Science degree in Nutrition in May 2018. She intends to pursue a career as a Registered Dietitian.