

Full Length Research Paper

Various sources of animal protein intake and their association with muscle mass index and insulin resistance in overweight postmenopausal women

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Many epidemiological studies have observed a positive relationship between animal protein intake (API) and the risk of type 2 diabetes (T2D). However, animal proteins are important in the aging population. Forty sedentary and healthy postmenopausal women were recruited in this study. Body composition (dual X-ray absorptiometry method, DXA), 3-d dietary record (API) and insulin resistance (homeostasis model assessment, HOMA) were assessed. Partial correlations were used to examine the relationship between total API (g/day) on muscle mass index (MMI) and HOMA. MMI ($r=0.408$; $p<0.01$) was associated to total API. Our results indicate that promoting an increase in animal proteins is important to maintain muscle mass in postmenopausal women.

Key words: Postmenopausal women, animal proteins, processed meats, insulin resistance, muscle mass.

INTRODUCTION

The high intake of animal protein in the Western diet (characterized by a high intake of red meat, processed meat, and high fat dairy products) may be directly or indirectly related to the occurrence of insulin resistance through different mechanisms (Peppas et al., 2002; Schulze et al., 2003; van Dam et al., 2002). To begin, animal proteins are divided into two types of meat, which are clearly differentiated by their color when raw, white or red meat. Red meat includes beef, pork, game (hunted for sport) and some fowl (ducks). Red meat is an important source of saturated fats, which could increase the risk of obesity (French et al., 1994) and cardiovascular disease (Fraser, 1999). On the other hand, white meats have been associated with a more favorable glycemic control and overall health (Montonen et al., 2005a; Montonen et al., 2005b; Villegas et al., 2003). Processed meats (animal proteins with additives

for longer storage duration: bacon, sausage, deli) contain nitrites and nitrates, which are toxic for the hepatic beta cells (Wolff, 1993). Whether red meat alone is actually associated with the risk of the development of type 2 diabetes (T2D) is not clearly understood or if this is mostly due to its combination with a unhealthy lifestyle such as high process meat intake (Aune et al., 2009) remains unclear.

Nevertheless, because they contain all essential amino acids and leucine, animal proteins have been suggested to be associated with the maintenance of muscle mass and function in older adults (Bartali et al., 2006; Pannemans et al., 1998). Sarcopenia, which is defined as the loss of muscle mass (Roubenoff and Hughes, 2000) and dynapenia, which is the loss of muscle strength (Clark and Manini, 2010; Roubenoff, 2000) during normal aging are risk factors for functional independence (Janssen, 2006) and frailty in elderly people (Lang et al., 2009). It has been proposed that an adequate total protein intake for elderly individual should sum up to 1.25 g/kg of body weight, instead of the current recommendation of 0.8 g/kg of body weight (Wolfe et al., 2008). Though meaningful, this has been mainly based

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on studies using essential amino acids rather than animal proteins from foods. Intriguingly, our laboratory (Lord et al., 2007) and others (Houston et al., 2008) showed a close relationship between animal protein intake and muscle mass, but did not address the potential impact on glucose metabolism. Based on the aforementioned associations between red meat intake and T2D, it can be speculated that while effective in maintaining muscle mass, animal proteins may be deleterious to glucose metabolism.

The objective of this study was to evaluate if some subclasses of animal proteins can be found beneficial for muscle mass without increasing the risk of T2D in obese postmenopausal women. Our hypothesis is that muscle mass index is related positively with red meat intake and white meat intake but processed meats with homeostasis model assessment (HOMA) value.

MATERIALS AND METHODS

Subjects

Forty healthy overweight postmenopausal women aged between 48 and 69 years were recruited by the use of advertisements in local newspapers to participate in an intervention study. Participants had to meet certain inclusion criteria, as assessed by phone interview: (1) healthy, (2) without major physical disability, (3) non-smoker, (4) non-regular exercisers, (5) moderate drinker (1 alcoholic beverage per day), (6) body mass index (BMI >27 kg/m²), (7) stable weight (± 2 kg) and (8) no medication that could influence metabolism (example: beta-adrenergic blocking agents, hypoglycemics, etc). Thereafter, the nature and goals of the study were thoroughly explained to the subjects and written informed consent was provided to them. All procedures were approved by the Ethics Committee of the Sherbrooke University Geriatric Institute.

Experimental procedures

After screening, participants were invited for a visit at the metabolic unit of the Research Centre on Aging of the Sherbrooke University Geriatric Institute. Participants were fasted for 12 h upon arrival at the metabolic unit. We measured body composition by dual X-ray absorptiometry method (DXA: GE, Prodigy Lunar, Madison, WI) (BMI, muscle mass index (MMI) kg fat free mass/m²) and proceeded with a 12 h fasting of glucose and insulin samples (HOMA). Instructions were then given for the completion of the 3 day dietary record and women were asked to return the dietary record one week later. To measure total and animal protein intakes, white meats (e.g. chicken, fish), red meats (e.g. beef, pork), processed meats (e.g. hot dogs) and other, such as eggs, milk and any other meat that did not fit in the aforementioned types. Subjects completed the physical activity scale for the elderly to control for physical activity (PASE).

Body composition

Body weight was measured using an electronic scale (± 0.2 kg SECA707, Hamburg, Germany). Standing height was measured using a wall stadiometer (Takei, Tokyo, Japan). BMI was measured as: body weight (kg)/height (m²). Fat mass (FM) and fat-free mass (FFM) were measured using a DXA (GE Prodigy Lunar Radiation

Corp, Madison, WI). In our laboratory, test-retest measures of FM and FFM in ten adults, with a 1-week interval, yielded a mean absolute coefficient of variation of 3.9 and 1.1%, respectively. MMI is generally used as an index of sarcopenia and is calculated as follows: total FFM (kg)/height (m²).

Dietary intake

Diets were recorded during 3 consecutive days including one weekend day. It has been demonstrated that a 3-day dietary record is valid to estimate dietary intakes in older adults without cognitive impairments (Luhmann et al., 1999). Each subject was instructed to maintain normal dietary habits throughout the period of data collection. Subjects were provided with a 5 kg food scale and instructed on how to complete a 3-day dietary record. When returned, we analyzed the journal with the participant to ensure validity of the entries. Total proteins were divided as animal and vegetable proteins. Animal proteins intake included all proteins from animal sources (red and white meat, eggs, fish and milk products). Dietary analyses were completed by using CANDAT SYSTEM, version 6.0 software (Candat, London, ON, Canada) to determine daily energy, protein, carbohydrate and lipid intakes.

Insulin resistance

Blood samples were collected after a 12 h overnight fast and a 15 min rest when participants were in a sitting position. Plasma glucose and insulin level were analyzed in the clinical laboratory of the Geriatric Institute. Insulin resistance based on the HOMA index was evaluated according to the following equation (Matthews et al., 1985): insulin (μ UI/ml) \times glucose (mmol/L)/22.5. HOMA shows a strong correlation with insulin sensitivity measured using the hyperinsulinemic-euglycemic clamp technique ($r = 0.88$) (Geloneze and Tambascia, 2006). A higher HOMA index indicates a poorer glucose metabolism.

Statistical methods

Values in the text and tables are presented as mean \pm standard deviation (SD). Partial correlations were used to examine the relationship between MMI and protein intake. Analysis covariance (ANCOVA) was used to examine differences between tertile groups of total animal protein intake for HOMA. Since age, physical activity, fat mass and total protein intake can affect glucose metabolism and MMI, they were all used as covariates. Significance was accepted at $p \leq 0.05$. All analyses were performed using Statistical Package for Social sciences (SPSS) (version 17.0; Chicago, IL, USA).

RESULTS

Baseline characteristics of the population are presented in Table 1. Partial correlations indicated that total animal protein intake was significantly and positively related to MMI when adjusted for physical activity, age, fat mass and total protein intake ($r=0.408$, $p<0.01$) and HOMA ($r=0.337$, $p<0.05$) (Table 2), when controlling for physical activity, age, fat mass and total protein intake. Fasting insulin but not glucose was also significantly and positively related to animal protein intake ($r=0.352$, $p=0.035$).

Table 1. Baseline characteristics of subjects.

Characteristic	Mean	SD
Age (years)	58.5	5.1
BMI (kg/m ²)	31.1	3.5
Muscle mass (kg)	40.4	4.1
MMI (kgMM/m ²)	16.1	1.7
Fasting glucose (mmol/L)	5.06	0.55
Fasting insulin (pmol/L)	64.2	33.07
Dietary intake (kcal)	2006	506
Total lipid intake (g)	83.4	33.7
Total protein intake (g)	85.2	22.4
Total carbohydrate intake (g)	203	67.9
Animal protein intake (g)	46.8	22.5
Vegetable protein intake (g)	38.4	11.9

Table 2. Partial correlations between protein intakes and MMI or HOMA, controlling for fat mass, age, physical activity, other meats and total protein intake.

Protein intake		MMI (kgFFM/m ²)	HOMA	Fasting insulin (pmol/L)	Fasting glucose (mmol/L)
Animal protein intake (n = 35)	R	0.408	0.337*	0.352*	0.097
	p	0.014	0.044	0.035	0.57
Red meat	R	-0.09	-0.2	-0.16	-0.212
	p	0.623	0.242	0.36	0.221
White meat	R	0.06	0.06	0.03	0.16
	p	0.713	0.738	0.88	0.366
Processed meats	R	0.23	0.34*	0.29	0.23
	p	0.18	0.05	0.08	0.174

*Statistically significant at $p < 0.05$.

Table 3. Relationship between total fiber intake and total animal protein, processed meats and red meat intake.

Parameter		Animal protein intake	Processed meats intake	Red meat intake
Total fiber intake (g)	R	0.076	-0.340*	-0.08*
	p	0.64	0.03	0.623

*Statistically significant at $p < 0.05$.

When dividing total animal protein into different types of meat (red, white, processed or others), only processed meats was positively associated with HOMA ($r=0.34$, $p < 0.05$), but not white or red meat, no associations were observed for muscle mass (Table 3).

Furthermore, we investigated if different types of meat were related with total fiber intake, which could help us indicate eating habits in our population. As such, processed meat intake was significantly and negatively related with total fiber intake ($r=-0.34$, $p < 0.05$).

Moreover, red meat intake was not related with total fiber intake ($r=-0.08$, $p=0.62$).

DISCUSSION

In this cross-sectional study, we sought to determine if animal protein intake showed significant associations with insulin resistance (HOMA) as well as MMI in postmenopausal women, and which type of meat has an

impact on these variables. The results of our study indicate that a greater intake in total animal proteins is significantly and positively related to fasting insulin levels, HOMA and muscle mass index. However, when dividing the types of meat into three types, processed meats was the only type associated positively with HOMA. Therefore, while total animal protein intake is related to the maintenance of muscle mass, only processed meats increases the risks of insulin resistance.

The results of this study are in agreement with the literature, indicating that high consumers of processed meats are at risk of insulin resistance and T2D (Aune et al., 2009; Montonen et al., 2005b; van Dam et al., 2002). These studies imply that it is not red meat per se, but the association with poor eating habits that is related with the incidence of T2D. Hence, our study seems to reconsider these assumptions by separating the different types of meat and demonstrate that processed meats and not red meats per se has an impact on insulin resistance parameters and that poor eating habits (high intake of processed meats, low total fiber intake) are also related with insulin resistance.

Animal proteins, which include red meat, are a good choice in the context of a healthy diet, because they contain all the essential amino acids, particularly leucine, which regulates muscle protein synthesis (Norton and Layman, 2006). They have been identified as a key nutrient to maintain muscle mass in older adults, but most studies examined essential amino acids supplements rather than proteins from foods (Symons et al., 2007; Volpi et al., 2003).

Interestingly, although counterintuitive, some data indicate that an elevated muscle mass may be associated with some impairments in insulin sensitivity in older adults (Brochu et al., 2008; Goulet et al., 2007). Our results suggest that animal protein intake may be a modulator of the association between muscle mass and glucose metabolism, explaining why a greater muscle mass would be associated with impaired glucose metabolism. Nevertheless, our results indicate that it may be processed meats that contribute to impairments in glucose metabolism. Based on these findings, it seems that protein animals should be recommended to older adults as long as they are educated to select healthy animal proteins such as unprocessed red or white meat and fish. Further studies on additional animal proteins sources such as eggs and milk produce should be conducted to determine if they can also be recommended in that context.

There are some limits to our study. The cross-sectional design of this study and the use of secondary analyses prevent the determination of cause-and-effect relationships. In this sense, we only had access to muscle mass and did not have measures of muscle quality. Also, HOMA is related to central insulin resistance. It would be of interest to measure peripheral glucose homeostasis with the euglycemic-hyperinsulinemic clamp. Furthermore, we did not

measure intramuscular lipids in muscle, which could help explain if there is a relationship between low muscle mass and impaired insulin sensitivity. Lastly, the positive relationship between protein intake and hyperinsulinemia is known to be a normal response when there is a high concentration of amino acids in the bloodstream, favoring a better glucose disposal (Manders et al., 2005). However, in our study, fasting glucose was not different between groups.

To conclude, our results suggest that while animal protein intake is favorable for obese postmenopausal women to preserve muscle mass, only processed meats is related to insulin resistance. Although, additional studies need to be conducted with regards to long term high intake of animal proteins on muscle mass and the risk of developing insulin resistance. This study provides interesting data on the intake on animal proteins with regards to the prevention of sarcopenia.

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