

学校编码: 10384

分类号_____密级_____

学号: 32620121150536

UDC _

厦门大学

硕士学位论文

红肉、膳食 Neu5Gc 摄入与机体 anti-Neu5Gc 抗体关系研究

Study on the relationship between red meat, dietary Neu5Gc intake and anti-Neu5Gc antibody

范杏丹

| | |
|---------|----------|
| 指导教师姓名: | 李红卫 副教授 |
| 专业名称: | 营养与食品卫生学 |
| 论文提交日期: | 2015年5月 |
| 论文答辩时间: | 2015年5月 |

2015年5月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为()课题(组)的研究成果,获得()课题(组)经费或实验室的资助,在()实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

年 月 日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

1.经厦门大学保密委员会审查核定的保密学位论文，于
年 月 日解密，解密后适用上述授权。

2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年 月 日

目 录

| | |
|-------------------------------|-----|
| 摘 要..... | I |
| Abstract..... | IV |
| 缩 写 词 表 | VII |
| 第一章 前言 | 1 |
| 1.1 Neu5Gc 概述..... | 1 |
| 1.2 机体中 Neu5Gc 的来源与吸收..... | 3 |
| 1.3 Neu5Gc 的生理功能..... | 4 |
| 1.4 Neu5Gc 与肿瘤 | 5 |
| 1.5 炎症与肿瘤 | 6 |
| 1.6 炎症因子与肿瘤 | 10 |
| 1.6.1 C 反应蛋白..... | 10 |
| 1.6.2 白细胞介素-6 | 11 |
| 1.7 唾液酸的检测方法 | 13 |
| 1.7.1 分光光度法 | 13 |
| 1.7.2 高效薄层色谱法 | 15 |
| 1.7.3 高效阴离子交换色谱-脉冲安培检测法 | 15 |
| 1.7.4 高效液相色谱法 | 15 |
| 1.7.5 液相色谱-质谱法 | 16 |
| 1.8 红肉、Neu5Gc、炎症与肿瘤的研究意义..... | 16 |
| 第二章 材料与方 法 | 18 |
| 2.1 常见食物唾液酸检测 | 18 |
| 2.1.1 试剂与材料 | 18 |
| 2.1.2 仪器与设备 | 18 |
| 2.1.3 色谱检测条件 | 18 |

| | |
|--|----|
| 2.1.4 样品的制备与处理 | 21 |
| 2.1.5 唾液酸的定性和定量 | 21 |
| 2.2 膳食唾液酸调查 | 21 |
| 2.3 Anti-Neu5Gc 抗体检测 | 21 |
| 2.4 C 反应蛋白检测..... | 22 |
| 2.5 白细胞介素-6 检测 | 22 |
| 2.6 统计分析方法 | 23 |
| 第三章 结果与分析 | 24 |
| 3.1 常见食物唾液酸检测 | 24 |
| 3.1.1 线性实验 | 24 |
| 3.1.2 精密度实验 | 24 |
| 3.1.3 重复性实验 | 24 |
| 3.1.4 稳定性实验 | 24 |
| 3.1.5 回收率实验 | 24 |
| 3.1.6 最低检出限 | 25 |
| 3.1.7 食物中的唾液酸含量 | 25 |
| 3.2 膳食 Neu5Gc 负荷..... | 27 |
| 3.2.1 调查对象基本情况 | 27 |
| 3.2.2 各类食物摄入情况 | 28 |
| 3.2.3 膳食 Neu5Gc 摄入情况..... | 30 |
| 3.3.1 Anti-Neu5Gc 抗体水平 | 32 |
| 3.3.2 Anti-Neu5Gc 抗体水平与膳食 Neu5Gc 摄入量的相关性分析 | 33 |
| 3.3.3 Anti-Neu5Gc 抗体水平与饮食的相关性分析 | 35 |
| 3.4 C 反应蛋白 (CRP) 水平分析 | 36 |
| 3.4.1 CRP 水平 | 36 |
| 3.4.2 CRP 水平与膳食 Neu5Gc 摄入量的相关性分析 | 36 |
| 3.4.3 CRP 水平与 anti-Neu5Gc 抗体水平的相关性分析 | 37 |
| 3.5 白细胞介素-6 (IL-6) 水平分析 | 37 |
| 3.5.1 白细胞介素-6 的水平 | 37 |

| | |
|---|----|
| 3.5.2 IL-6 与膳食 Neu5Gc 摄入量的相关性分析 | 38 |
| 3.5.3 IL-6 水平与 anti-Neu5Gc 抗体水平的相关性分析 | 39 |
| 第四章 讨论与结论 | 40 |
| 参考文献..... | 43 |
| 附 录 1..... | 55 |
| 附 录 2..... | 59 |
| 致 谢..... | 60 |

厦门大学博硕士论文摘要库

Contents

| | |
|--|------------|
| Chinese abstract | I |
| English abstract | IV |
| Abbreviations | VII |
| Chapter 1 Preface | 1 |
| 1.1 Review of Neu5Gc | 1 |
| 1.2 Source and absorption of human Neu5Gc | 3 |
| 1.3 Physiological function of Neu5Gc | 4 |
| 1.4 Neu5Gc and tumor | 5 |
| 1.5 Inflammation and tumor | 6 |
| 1.6 Inflammatory factors and tumor | 10 |
| 1.6.1 CRP | 10 |
| 1.6.2 IL-6 | 11 |
| 1.7 Methods of determining sialic acid | 13 |
| 1.7.1 Spectrophotometry | 13 |
| 1.7.2 High performance thin layer chromatography | 15 |
| 1.7.3 High performance anion-exchange chromatography with pulse amperometric detector | 15 |
| 1.7.4 High performance liquid chromatography..... | 15 |
| 1.7.5 Liquid chromatography tandem mass spectrometry | 16 |
| 1.8 Perspective of red meat, Neu5Gc, inflammation and tumor | 16 |
| Chapter 2 Materials and Methods | 18 |
| 2.1 Determination of sialic acid in foods | 18 |
| 2.1.1 Regent and materials..... | 18 |
| 2.1.2 Instruments and equipment..... | 18 |
| 2.1.3 Chromatographic condition | 18 |
| 2.1.4 Preparation of food samples..... | 21 |

| | |
|---|-----------|
| 2.1.5 Qualitative and quantitative determination of sialic acid | 21 |
| 2.2 Dietary sialic acid survey..... | 21 |
| 2.3 Detection of Anti-Neu5Gc antibody | 21 |
| 2.4 Detection of CRP..... | 22 |
| 2.5 Detection of IL-6 | 22 |
| 2.6 Methods of statistical analysis | 23 |
| Chapter 3 Results and analysis..... | 24 |
| 3.1 Determination of sialic acid in foods | 24 |
| 3.1.1 Results of linearity | 24 |
| 3.1.2 Precision results | 24 |
| 3.1.3 Repeatability results..... | 24 |
| 3.1.4 Stability results..... | 24 |
| 3.1.5 Recovery results..... | 24 |
| 3.1.6 Detection limit results | 25 |
| 3.1.7 Concentration of sialic acid in foods | 25 |
| 3.2 Evaluation of Neu5Gc intake | 27 |
| 3.2.1 General characteristics of subjects..... | 27 |
| 3.2.2 Intakes of foods..... | 28 |
| 3.2.3 Intake of Neu5Gc..... | 30 |
| 3.3.1 Anti-Neu5Gc antibody concentration | 32 |
| 3.3.2 Correlation of anti-Neu5Gc antibody and Neu5Gc intake | 33 |
| 3.3.3 Correlation of anti-Neu5Gc antibody and diet..... | 35 |
| 3.4 Analysis of CRP..... | 36 |
| 3.4.1 Concentration of CRP | 36 |
| 3.4.2 Correlation of CRP and Neu5Gc intake..... | 36 |
| 3.4.3 Correlation of CRP and anti-Neu5Gc antibody | 37 |
| 3.5 Analysis of IL-6 | 37 |
| 3.5.1 Concentration of IL-6 | 37 |
| 3.5.2 Correlation of IL-6 and Neu5Gc intake | 38 |

| | |
|--|-----------|
| 3.5.3 Correlation of IL-6 and anti-Neu5Gc antibody | 39 |
| Chapter 4 Discussion and conclusions | 40 |
| References | 43 |
| Appendix 1 | 55 |
| Appendix 2 | 59 |
| Acknowledgements | 60 |

厦门大学博硕士学位论文摘要库

摘要

唾液酸类化合物是以九碳糖神经氨酸(5-amino-3,5-dideoxy-D-glycero-D-galactononulosonic acid)为基本结构的一族衍生物的总称。目前,已分离和鉴定的有 50 余种^[1],包括 N-乙酰基神经氨酸(N-acetylneuraminic acid,Neu5Ac)、N-羟乙酰基神经氨酸(N-glycoulylneuraminic acid,Neu5Gc)和 2-酮基-3-脱氧九酮糖酸(2-keto-3-deoxy-nonulosonic acid,KDN)三种核心单体,其余的唾液酸均由这三种单体衍生而来。在人体组织中发现的唾液酸主要是 Neu5Ac, Neu5Gc 是 Neu5Ac 的羟基化产物,普遍存在于除人类以外的哺乳动物体内,如马、狗和大猩猩等。由于健康人体组织中编码 CMP-Neu5Ac 羟化酶的基因在进化过程中发生突变,故人体内无法合成 Neu5Gc^[2,3]。有研究表明,健康人体可从红肉及奶制品中获取外源性 Neu5Gc^[4]。

2007 年世界癌症研究基金会在其第二份《食物、营养、身体活动和癌症预防》报告中明确指出^[5],红肉和加工肉类的过多摄入与癌症的发生密切相关,红肉可能是导致某些肿瘤的原因之一。充分的流行病学证据表明红肉和加工肉类是导致结肠/直肠癌的因素之一;红肉是导致食管癌、肺癌、胰腺癌及子宫内膜癌的原因之一;加工肉类是导致食管癌、肺癌、胃癌及前列腺癌的原因之一。过多食用红肉和加工肉类会使患肺癌和直肠癌的风险增加 20%,会使患肠癌的风险增加 30%。

红肉是对某些肉品的总称,通常指红色肌肉纤维比白色肌肉纤维多的动物肉,包括牛肉、羊肉和猪肉等,及其加工制品。有研究人员发现了一种非人体产生的结构糖—Neu5Gc,随着摄取的红肉进入人体,使人体产生 anti-Neu5Gc 抗体,该免疫反应过程可导致慢性炎症,可能是导致癌症的机理之一^[6,7]。

本研究拟通过对食物中 Neu5Gc 含量的检测、人体 Neu5Gc 负荷的评价以及人体中 anti-Neu5Gc 抗体水平的检测,探讨摄入 Neu5Gc 是否会引起相应抗体水平的升高。而炎症因子 C 反应蛋白(C reactive protein, CRP)和白细胞介素-6(Interleukin-6, IL-6)是常见的炎症反应的指标,考察二者与膳食 Neu5Gc 摄入量及 anti-Neu5Gc 抗体水平的关系,可知 Neu5Gc 引起炎症反应是否与 CRP 及 IL-6 有关。目前,国内外均缺乏食物唾液酸含量的基础数据,人体唾液酸摄入及对机

体影响的评价更是少有研究。因此,本研究对国内常见食物唾液酸(Neu5Gc和Neu5Ac)的含量进行检测,评价人体Neu5Gc的负荷,并检测机体anti-Neu5Gc抗体水平、CRP水平及IL-6水平,以评估由膳食Neu5Gc的摄入量对人体可能的健康风险,进行合理的膳食指导。

研究内容:(1)于厦门市场采购人群食用频率较高的米面、禽肉、畜肉、水产品、奶类、豆类、蛋类、蔬菜水果等食物102种,用液相色谱仪-质谱联用法(liquid chromatography-mass spectrometry, LC-MS/MS)检测其中的Neu5Gc和Neu5Ac含量;(2)采取随机抽样的方法,在厦门市居民中抽取500人进行膳食调查,通过24h回顾法结合膳食史法获得其膳食摄入情况,分析膳食是否合理,并结合“食物-唾液酸含量”进行Neu5Gc负荷的评价;(3)采集被调查者的血液,分离血浆,检测其anti-Neu5Gc抗体浓度、CRP浓度及IL-6浓度;(4)用Spearman相关分析方法分析膳食Neu5Gc摄入量与anti-Neu5Gc抗体浓度、CRP浓度及IL-6浓度的相关性。

结果:(1)食物样品中检测出Neu5Ac或Neu5Gc的食物有蛋类、奶类、禽肉、畜肉和部分海鲜,其中总唾液酸含量最高的是蛋黄($1110.32 \pm 62.04 \mu\text{g/g}$),其次依次是蛋白($367.63 \pm 8.01 \mu\text{g/g}$)、奶类、红肉;Neu5Gc含量最高的是牛肉($30.34 \pm 2.80 \mu\text{g/g}$),其次依次是羊肉($20.38 \pm 4.67 \mu\text{g/g}$)、牛奶($10.87 \pm 1.5 \mu\text{g/mL}$)。(2)回收有效问卷为496份,496名研究对象的膳食Neu5Gc摄入量呈正偏态分布,最高值为83.900mg/d,最低值为0mg/d,中位数为4.619mg/d,男性平均水平为6.602mg/d,女性为3.837mg/d,且男女之间的差异存在统计学意义($P=0.000$),可以认为男性总体的Neu5Gc摄入水平高于女性。(3)研究对象的anti-Neu5Gc抗体水平成正偏态分布,最高值为12.805 $\mu\text{g/mL}$,最低值为0.676 $\mu\text{g/mL}$,平均水平为3.072 $\mu\text{g/mL}$;男性平均水平为3.170 $\mu\text{g/mL}$,女性为2.993 $\mu\text{g/mL}$,男女之间的差异存在统计学意义($P=0.000$),可以认为男性总体的anti-Neu5Gc抗体水平高于女性。(4)Neu5Gc摄入量与anti-Neu5Gc抗体浓度存在相关关系(全部研究对象 $r_s=0.222$, $P=0.000$;男性 $r_s=0.143$, $P=0.028$;女性 $r_s=0.305$, $P=0.000$),当Neu5Gc摄入量在0mg/d~8mg/d之间,anti-Neu5Gc抗体水平随之升高而升高,二者存在正相关关系。当Neu5Gc摄入量 $>8\text{mg/d}$,anti-Neu5Gc抗体水平反而随之升高而降低。根据本实验室对食物中Neu5Gc含

量的检测，8mg 的 Neu5Gc 相当于 2.637kg 的牛肉、3.925kg 的羊肉，或者 7.36L 的牛奶中的 Neu5Gc 含量，正常成人每日对诸类食物的摄入量远低于这一水平。

(5) 研究对象的 CRP 水平与膳食 Neu5Gc 的摄入量存在正相关关系（女性未发现相关）（全部研究对象 $r_s=0.102$, $P=0.024$ ；男性 $r_s=0.165$, $P=0.011$ ），与 anti-Neu5Gc 抗体水平未发现相关关系。（6）研究对象的 IL-6 水平与膳食 Neu5Gc 的摄入量存在正相关关系（全部研究对象 $r_s=0.126$, $P=0.005$ ；男性 $r_s=0.165$, $P=0.011$ ；女性 $r_s=0.123$, $P=0.049$ ），与 anti-Neu5Gc 抗体水平存在正相关关系（女性未发现相关）（全部研究对象 $r_s=-0.104$, $P=0.020$ ；男性 $r_s=-0.129$, $P=0.047$ ）。

结论：（1）食物中 Neu5Gc 含量较高的是红肉类和奶类；（2）Neu5Gc 摄入量在 0mg/d~8mg/d 之间，anti-Neu5Gc 抗体浓度与其存在正相关关系；（3）长期摄入 Neu5Gc，会升高机体内的 CRP 和 IL-6 水平，引发慢性炎症；（4）anti-Neu5Gc 抗体浓度与 CRP 未发现相关、与 IL-6 水平存在负相关，Neu5Gc 引起三者水平升高可能是通过不同的机制达到的。

从以上的结论我们认为，在一定范围内，过多摄入含有 Neu5Gc 的食物，如红肉或奶制品，会升高体内 anti-Neu5Gc 抗体的浓度，也会提高 CRP 和 IL-6 的水平，引发体内的慢性炎症，进而增加罹患肿瘤的风险。

关键词：唾液酸； Neu5Gc； CRP； IL-6； 红肉； 肿瘤

Abstract

Sialic acids (Sias) are a group of nine carbon monosaccharides with 5-amino-3,5-dideoxy-D-glycero-D-galactononulosonic acid as basic structure. At present, there are more than 50 kinds of Sia have been isolated and identified^[1], among which are N-acetylneuraminic acid, N-glycolylneuraminic acid and 2-keto-3-deoxy-nonulosonic acid, the three kinds of main monomer from which other Sias derive. Neu5Ac is the most common form of Sia in human, and is expressed ubiquitously throughout the human body. Neu5Gc is abundant in most mammals, but not in normal humans, because of the evolutionary loss of the gene encoding CMP-Neu5Ac hydroxylase(CMAH) that converts Neu5Ac into Neu5Gc^[2,3]. There is evidence that Neu5Gc can be metabolically incorporated into human glycoproteins by both normal and cancerous tissues due to the dietary availability of Neu5Gc, predominantly red meats and dairy products^[4].

According to the World Cancer Research Fund, excessive intake of meat, particularly red meat and processed meat, might closely related to cancers including rectal cancer, colon cancer, prostate cancer and breast cancer^[5]. Adequate epidemiological evidence suggests that, red meat and their processed product may increase the risk of lung cancer and rectal cancer by 20 percent, and enhance the risk of intestinal cancer by 30 percent.

Red meat refers to certain kinds of animal meat whose red muscle fiber is more than white muscle fiber, for example, beef, pork, lamb and their processed products. As to the health risk factor in red meat, opinions are divided. Of particular concern is the a kind of sialic acid produced inside human body after eating red meat, N-glycolylneuraminic acid (Neu5Gc). Some researches speculated that, the immune response caused by Neu5Gc and its corresponding antibody gives rise to chronic inflammation, which leads to cancer^[6,7].

This study intends to determine the Sia content in daily foods, evaluate the Neu5Gc load and detect the anti-Neu5Gc antibody concentration, to explore whether Neu5Gc

intake would increase the corresponding antibody. Among the indicators of inflammatory reaction, CRP and IL-6 are two of significance. Whether their concentration related to Neu5Gc intake and anti-Neu5Gc antibody level is also needed to explore. Currently, there are not data of Sia content in foods published, neither researches about the impact of intake Sia on human body. Therefore, it is of great significance to analyze the Sia speciation and content in foods. If we get to know the Sia content in Chinese conventional foods, and the relationship between the intake Neu5Gc and its corresponding antibody, as well as the level of inflammatory factors, we can adjust our diet to avoid the possible health risk brought about by the Sia-containing foods.

In this research, we completed the following work: a. Detected Neu5Ac and Neu5Gc content in 102 kinds of Chinese conventional foods by liquid chromatography-mass spectrometry (LC-MS/MS); b. Conducted dietary survey to 500 healthy adult urban residents in Xiamen selected randomly to get their intakes of Neu5Gc; c. Detected the concentration of anti-Neu5Gc antibody, CRP and IL-6 of the respondents; d. Analyzed the correlation between Neu5Gc intake and the concentration of anti-Neu5Gc antibody, CRP and IL-6.

Results: (1) The main source of dietary Neu5Gc are red meat and dairy, especially beef ($30.34 \pm 2.80 \mu\text{g/g}$), lamb ($20.38 \pm 4.67 \mu\text{g/g}$) and milk ($10.87 \pm 1.5 \mu\text{g/mL}$). (2) Effective questionnaires are 496, which shows that, the highest Neu5Gc intake is 83.900mg/d, the lowest is 0mg/d, average 4.619mg/d, and that of male is higher than female. (3) The concentration of anti-Neu5Gc antibody shows positive skewness, the highest of which is $12.805 \mu\text{g/mL}$, the lowest is $0.676 \mu\text{g/mL}$, average $3.072 \mu\text{g/mL}$. (4) Anti-Neu5Gc antibody level increases with the rising of Neu5Gc intakes when the latter is below 8mg/d, equaling to 263.68g beef, 392.54g lamb or 735.97mL milk. The Neu5Gc intakes correlated with the foods statistically, particularly beef and milk. (5) Both of CRP concentration and IL-6 concentration are positively correlated with Neu5Gc intake, while only IL-6 is correlated with the anti-Neu5Gc concentration, which is negatively.

Conclusion: (1) The most Neu5Gc-containing foods are red meat and dairy

products. (2) The concentration of anti-Neu5Gc antibody increases with the intake of Neu5Gc as the latter between 0mg/d~8mg/d. (3) Long-term Neu5Gc intake would increase the concentration of CRP and IL-6, giving rise to inflammation. (4) There isn't correlation found between anti-Neu5Gc antibody and CRP, while anti-Neu5Gc is negatively related to IL-6, which may result from their different mechanism.

It comes to a conclusion that, excessive intake of Neu5Gc-containing foods like red meat and dairy products would enhance the concentration of anti-Neu5Gc, CRP and IL-6, resulting in inflammatory response, increasing the risk of cancer.

Key words: Sialic acid; Neu5Gc; CRP; IL-6; red meat; cancer

缩写词表

| | |
|----------------|--|
| Neu5Ac | N-acetylneuraminic acid N-乙酰神经氨酸 |
| Neu5Gc | N-glycolyl neuraminic acid N-羟乙酰神经氨酸 |
| KDN | 2-keto-3-deoxy-nonulosonic acid 2-酮基-3-脱氧九酮糖酸 |
| CMP-Neu5Ac | Cytidine monophosphate N-acetylneuraminic acid 唾液酸胞苷单磷酸酯 |
| CMP-6-P | Cytidine monophosphate-6-phosphates 胞苷酸-6-磷酸 |
| CRP | C reactive protein C 反应蛋白 |
| IL-6 | Interleukin-6 白细胞介素-6 |
| LC-MS/MS | Liquid chromatography-mass spectrometry 液相色谱仪-质谱联用法 |
| Sia | Sialic acid 唾液酸 |
| CMP-SA | Cytidine monophosphate-Sialic acid 胞苷酸唾液酸 |
| TNF- α | Tumor necrosis factor alpha 肿瘤坏死因子 α |
| PDGF | Platelet derived growth factor 血小板源性生长因子 |
| EGF | Epidermal growth factor 表皮生长因子 |
| FGF | Fibroblast growth factor 成纤维细胞生长因子 |
| VEGF | Vascular endothelial growth factor 血管内皮生长因子 |
| ROS | Reactive oxygen species 活性氧簇 |
| RNS | Reactive nitrogen species 活性氮簇 |
| MIF | Migration inhibitory factor 巨噬细胞游走抑制因子 |
| EGFr | Epidermal growth factor receptor 表皮生长因子受体 |
| NF- κ B | Nuclear factor kappa B 转录核因子 κ B |
| MDSCs | Myeloid-derived suppressor cells 髓源抑制性细胞 |
| bF-GF | Basic fibroblast growth factor 碱性成纤维生长因子 |
| MMPs | Matrix metalloproteinase 基质金属蛋白酶 |

Degree papers are in the “[Xiamen University Electronic Theses and Dissertations Database](#)”.

Fulltexts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.