Research Article
Genetic and Metabolic Diseases

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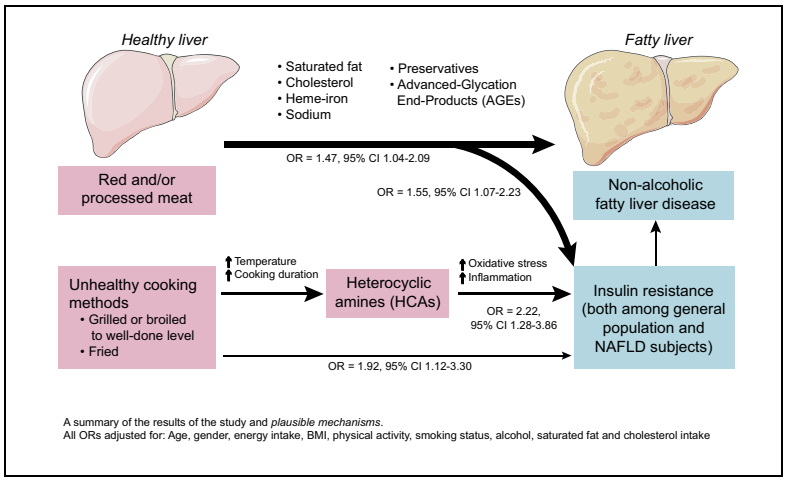
High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance

Graphical abstract



Highlights

- High consumption of red and/or processed meat is related to NAFLD and insulin resistance.
- Consumption of meat cooked in unhealthy methods is related to insulin resistance.
- Consumption of HCAs is related with insulin resistance.
- These associations are independent of saturated fat and cholesterol intake.
- If confirmed prospectively, limiting the consumption of unhealthy meats may be advised.

Authors

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Lay summary

High red and processed meat consumption is related to several diseases. In addition, cooking meat at high temperatures for a long duration forms heterocyclic amines, which have harmful health effects. Non-alcoholic fatty liver disease is a significant public health burden and its formation is strongly related to insulin resistance. In this study, both were found to be more frequent in people who consume relatively high quantities of red and processed meat. In addition, a high intake of heterocyclic amines was associated with insulin resistance.

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High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance

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Background & Aims: High red and processed meat consumption is related to type 2 diabetes. In addition, cooking meat at high temperatures for a long duration forms heterocyclic amines (HCAs), which are related to oxidative stress. However, the association between meat consumption and non-alcoholic fatty liver disease (NAFLD) is yet to be thoroughly tested. Therefore, we aimed to test the association of meat type and cooking method with NAFLD and insulin resistance (IR).

Methods: This was a cross-sectional study in individuals who were 40–70 years old and underwent screening colonoscopy between 2013 and 2015 in a single center in Israel. NAFLD and IR were evaluated by ultrasonography and homeostasis model assessment. Meat type and cooking method were measured by a food frequency questionnaire (FFQ) and a detailed meat questionnaire. Unhealthy cooking methods were considered as frying and grilling to a level of well done and very well done. Dietary HCA intake was calculated.

Results: A total of 789 individuals had a valid FFQ and 357 had a valid meat questionnaire. High consumption of total meat (portions/day above the median) (odds ratio [OR] 1.49; 95% CI 1.05–2.13; $p = 0.028$; OR 1.63; 1.12–2.37; $p = 0.011$), red and/or processed meat (OR 1.47; 95% CI 1.04–2.09; $p = 0.031$; OR 1.55; 1.07–2.23; $p = 0.020$) was independently associated with higher odds of NAFLD and IR, respectively, when adjusted for: body mass index, physical activity, smoking, alcohol, energy, saturated fat and cholesterol intake. High intake of meat cooked using unhealthy methods (OR 1.92; 95% CI 1.12–3.30; $p = 0.018$) and HCAs (OR 2.22; 95% CI 1.28–3.86; $p = 0.005$) were independently associated with higher odds of IR.

Conclusion: High consumption of red and/or processed meat is associated with both NAFLD and IR. High HCA intake is associated with IR. If confirmed in prospective studies, limiting the consumption of unhealthy meat types and improving preparation methods may be considered as part of NAFLD lifestyle treatment.

Lay summary: High red and processed meat consumption is related to several diseases. In addition, cooking meat at high temperatures for a long duration forms heterocyclic amines, which have harmful health effects. Non-alcoholic fatty liver disease is a significant public health burden and its formation is strongly related to insulin resistance. In this study, both were found to be more frequent in people who consume relatively high quantities of red and processed meat. In addition, a high intake of heterocyclic amines was associated with insulin resistance.

Introduction

Non-alcoholic Fatty liver disease (NAFLD) is becoming a major global health burden in both developed and developing countries.[1] NAFLD is considered as the hepatic component of the metabolic syndrome, with insulin resistance (IR) as the key factor in its pathophysiology.[2] Unhealthy Western lifestyle plays a major role in the development and progression of NAFLD,[3] namely, lack of physical activity and high consumption of fructose and saturated fat.[4,5] There are other common foods in the Western diet, namely red and processed meats, which may also increase the risk for NAFLD.[6] Meat in general contains valuable nutrients for human health including protein, iron, zinc and vitamin B12.[7] However, meat also contains saturated fatty acids (SFA) and cholesterol, both harmful for patients with NAFLD,[8–11] as well as other potentially harmful compounds such as heme-iron,[12] sodium,[13] other preservatives[12] and advanced glycation end products (AGEs).[12,14] Indeed, high meat consumption has been demonstrated to be associated with IR and type 2 diabetes,[15–17] the metabolic syndrome[17] and oxidative stress.[18] More specifically, red meat has been shown to be associated with a higher risk of mortality, owing to chronic liver disease and hepatocellular carcinoma.[19] The association between meat consumption and NAFLD was demonstrated in a few studies,[5,6,20,21] in which meat type and cooking method were not fully addressed. We have previously demonstrated an independent association between high meat consumption and NAFLD,[20] with no distinction between meat types or cooking methods, because of a small sample size and lack of information on the cooking methods in the standard food frequency questionnaire (FFQ).

Meat cooking methods have clinical significance, as unfavorable heterocyclic amines (HCAs) may be formed during

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cooking,[22] as a result of the interaction between creatine, amino acids and sugars, especially in protein-rich foods cooked under dry conditions, high temperature (above 150 °C) and prolonged cooking time.[5,7,23,24] The quantity of HCAs in cooked meat is affected by meat type, cooking method, cooking time and temperature.[23,25] In general, frying, broiling and grilling are methods that produce a greater quantity of HCAs.[25] These compounds have been extensively demonstrated to be associated with some types of cancer.[23] A positive association between HCA intake and increased oxidative stress has been demonstrated *in vitro*[26] and in human studies, implying that it may consequently increase the risk of chronic diseases.[27] However, human studies testing the association of dietary HCAs with IR and NAFLD are lacking. Therefore, the current study aimed to assess the independent association of meat type, cooking method and HCA intake with NAFLD and IR in a large well characterized general population sample.

Materials and methods

Study design and population

This is a cross-sectional study among individuals who underwent screening colonoscopy at the Department of Gastroenterology and Hepatology in the Tel Aviv Medical Center, and agreed to participate in a metabolic and hepatic screening study between the years 2013 and 2015. Exclusion criteria included: presence of HBsAg or anti-HCV antibodies, fatty liver suspected to be secondary to hepatotoxic drugs, inflammatory bowel disease, celiac disease and excessive alcohol consumption (≥30 g/day in men or ≥20 g/day in women).[3,28] In addition, individuals who reported an unreasonable caloric intake were excluded: below or above the acceptable range for men 800–4,000 kcal/day and for women 500–3,500 kcal/day.[29]

The study was approved by the medical center IRB committee and all patients signed an informed consent.

Data collection

Study participants were invited for a single day visit, in which they underwent fasting blood tests, liver ultrasound, a face-to face interview using a structured questionnaire, assembled by the Israeli Ministry of Health and used in national surveys,[30] including demographic details, health status, alcohol consumption, smoking and physical activity habits. In addition, they completed a FFQ and specific validated meat consumption and cooking method questionnaire.[31] To avoid reporting bias, the participants were informed of their abdominal ultrasonography (AUS) and blood tests results only after completing the questionnaires.

Fatty liver was diagnosed by AUS using standardized criteria, and performed in all individuals with the same equipment (EUB-8500 scanner Hitachi Medical Corporation, Tokyo, Japan) and by the same experienced radiologist (Webb M) as previously described.[20] The ratio between the brightness level of the liver and the right kidney was calculated to determine the hepato-renal index (HRI), which has been previously validated against liver biopsy.[32]

IR was evaluated by high homeostasis model assessment (HOMA) score, defined by the 75th percentile (upper quartile, Q4) of the study sample HOMA levels, as accepted[33] (corresponding to a value >3.31 in the total sample and >2.97 in the meat questionnaire sub-sample). This method was chosen given the lack of a standardized cut-off, because of interlaboratory variation in insulin measurements and population-specific values.[2]

Type 2 diabetes was defined as fasting glucose ≥126 mg/dl and/or glycated hemoglobin (HbA1C) ≥6.5% and/or use of diabetic medications.[34] Since insulin concentrations may start to decline in patients with diabetes,[2] those with diabetes who had no IR according to upper quartile of HOMA levels (n = 44) were considered as having IR.

Nutritional variables evaluation and definitions

The semi-quantitative FFQ, which was assembled by the Food and Nutrition Administration, Ministry of Health and tailored to the Israeli population, is composed of 117 food items with specified serving sizes. For each food item, participants indicated their average frequency of consumption over the past year in terms of the number of specified serving sizes consumed per day/week/month, less than once a month, or never. The nutrient components of each food item were taken from the Israeli national nutrient database (BINAT), Food and Nutrition Administration, Ministry of Health. Meat types were categorized as accepted.[35] The epidemiologic literature usually classifies the meat consumed as "red", "white", and "processed". All meats obtained from mammals are red meats because they contain more myoglobin than white meat (obtained from chicken).[36] *Total red meat* was composed of: beef steak or roast, beef internal organs, fried beef patties, lamb and pork.[35] *Processed meat* included: hamburger, salami, pastrami, sausages, processed schnitzel and canned meat, according to the following definition: meat that has been transformed through salting, smoking, or other processes to enhance flavor or improve preservation.[35] *Total meat* was composed of all the above categories plus chicken and turkey (unprocessed), excluding fish and seafood. The median consumption of each meat category was used as a cut-off to define a population-specific high consumption, as accepted in epidemiological studies.[37]

In order to elaborate on the association with the meat cooking method, a validated meat questionnaire[31] was used to evaluate specific cooking methods and degree of cooking. Cooking methods included: grilling, frying, broiling, and stewing.[7,24] Degree of cooking included the following categories: very well done, well done, medium well, rare.[31] We defined meat cooked by "unhealthy methods" as all types of meat grilled or broiled to a level of well done and very well done, or fried (in weekly portions).

The quantity of HCAs was calculated for all meat types combined (ng/day), based on the meat consumption questionnaire and analyzed by the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) software,[38] used for epidemiological research.[39,40] The meat questionnaire was added at a later stage of the study and thus performed on a sub-sample.

Statistical analysis

Statistical analyses were performed using SPSS version 23 (IBM-SPSS Armonk, NY) software. Continuous variables are presented as means ± SD. To test differences in continuous variables between two or three groups, the independent samples *t* test and ANOVA were performed, respectively. Associations between nominal variables were performed with the Pearson Chi-Square test. In both Chi-Square test and ANOVA, *p* for trend was calculated when needed.

A multivariate logistic regression analysis was performed to test the adjusted association between meat intake and NAFLD or IR, adjusting for potential confounders (NAFLD-related variables which were different between the meat intake categories as presented [Table 1]) or mediators (saturated fat and cholesterol intake). Odds ratio (OR) and 95% confidence interval (CI) CIs are presented. In addition, a multivariate linear regression for the continuous outcome of HOMA levels (log-transformed to achieve normal distribution), with the same covariates was performed. *p* values of <0.05 were considered statistically significant for all analyses.

For further details regarding the materials used, please refer to the CTAT table.

Results

Description of the study population and comparison between high and low (by median) meat eaters

Out of 970 individuals who participated in the study, 933 completed all tests (*i.e.* blood test and AUS), 18 were excluded because of viral liver disease and five because of alcohol abuse. Individuals with unreasonable reported caloric intake[29] were excluded (57 women and 64 men). Finally, 789 individuals were included in the main analysis, and of those, a sub-sample of 357 completed the meat questionnaire (Fig. 1).

In the entire sample, 52.60% were men, the proportion of participants with type 2 diabetes was 14.8% (n = 117), mean age was 58.83 ± 6.58 years and mean body mass index (BMI) was 28.54 ± 5.43 kg/m^2 (the meat questionnaire sub-sample had very similar characteristics). NAFLD was diagnosed in 38.70% of participants, and IR was diagnosed in 30.5% (34.2% and 28.5% in the meat questionnaire sub-sample, respectively). The proportion of red and white meat intake was about 1/3 and 2/3 respectively, which are similar to the usual intake of the

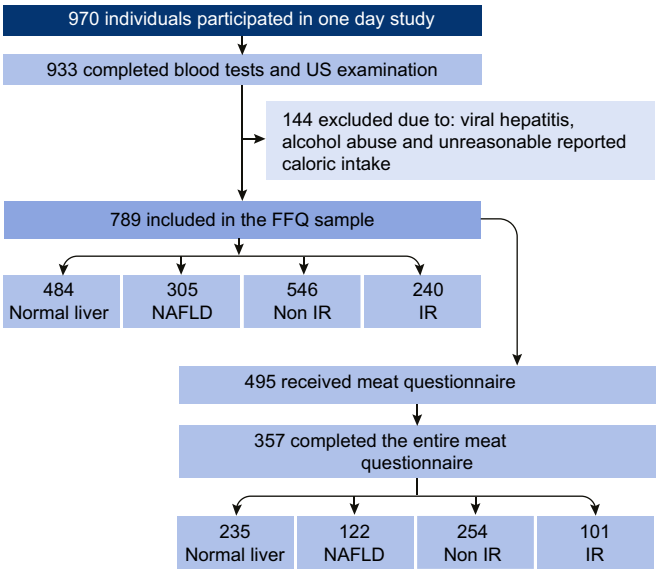

Fig. 1. Flow-chart of the study population. Three people in the FFQ sample and two people who completed the entire meat questionnaire did not have an insulin test. FFQ, food frequency questionnaire; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; SFA, saturated fatty acids.

Table 1. Description of the study population and comparison between high and low (by median) meat eaters (mean ± SD, unless otherwise stated).

Parameter	Total meat			Red meat and/or processed meat		
	Daily portions <1.10 n = 394	Daily portions ≥1.10 n = 395	*p* value	Daily portions <0.33 n = 391	Daily portions ≥0.33 n = 398	*p* value
Age (years)	59.29 ± 6.03	58.38 ± 7.06	0.052	59.40 ± 6.09	58.27 ± 6.98	0.016
Gender (male)%	42.40	62.80	<0.001	41.40	63.60	<0.001
BMI (kg/m^2) (20–25)	27.61 ± 5.35	29.46 ± 5.35	<0.001	27.62 ± 5.12	29.45 ± 5.57	<0.001
Waist circumference (cm)	97 ± 14	103 ± 13	<0.001	97 ± 14	102 ± 13	<0.001
NAFLD%	30.70	46.60	<0.001	31.20	46.00	<0.001
HRI (score)	1.35 ± 0.43	1.50 ± 0.49	<0.001	1.37 ± 0.45	1.47 ± 0.48	0.003
High HOMA-IR (above 4th quartile*)%	23.00	38.10	<0.001	23.40	37.50	<0.001
HOMA-IR (score)	2.49 ± 1.72	3.48 ± 4.99	<0.001	2.51 ± 1.76	3.45 ± 4.96	<0.001
Glucose (mg/dl) (<100)	87.76 ± 17.26	93.08 ± 25.24	0.001	87.95 ± 17.18	92.85 ± 25.59	0.001
HbA1C (%)	5.81 ± 0.64	5.94 ± 0.86	0.016	5.81 ± 0.68	5.94 ± 0.83	0.022
Type-2 diabetes	12.20	17.50	0.037	11.50	18.10	0.009
Total Chol (mg/dl) (<200)	185.41 ± 36.32	177.81 ± 34.43	0.003	185.47 ± 36.58	177.81 ± 34.17	0.002
Triglycerides (mg/dl) (<150)	109.27 ± 63.25	122.68 ± 69.75	0.005	108.27 ± 59.29	123.56 ± 72.84	0.001
ALT (U/L) (5–39)	24 ± 10	28 ± 17	<0.001	24 ± 14	27 ± 15	0.003
AST (U/L) (7–40)	24 ± 7	25 ± 9	0.274	25 ± 8	25 ± 9	0.927
GGT (U/L) (6–28)	26 ± 22	31 ± 32	0.013	26 ± 25	30 ± 29	0.063
Uric acid (mg/dl)	5.28 ± 1.40	5.65 ± 1.28	<0.001	5.29 ± 1.36	5.63 ± 1.32	0.001
CRP (mg/L) (<5)	3.49 ± 5.99	3.97 ± 5.33	0.236	3.57 ± 6.15	3.89 ± 5.16	0.440
Energy intake (kCal/day)	1840.21 ± 685.53	2211.39 ± 664.22	<0.001	1908.18 ± 692.68	2141.81 ± 687.80	<0.001
Saturated fat intake (% of daily kCals)	12.17 ± 3.89	12.59 ± 3.45	0.108	11.74 ± 3.77	13.00 ± 3.48	<0.001
Cholesterol intake (mg/day)	261.24 ± 143.98	408.75 ± 207.87	<0.001	277.78 ± 152.75	391.38 ± 211.85	<0.001
Sugared beverages (portions/day)	2.01 ± 3.34	2.01 ± 3.29	1.000	2.19 ± 3.47	1.84 ± 3.15	0.138
Fiber intake (g/day)	23.03 ± 12.91	23.42 ± 10.41	0.637	23.47 ± 12.81	22.98 ± 10.55	0.557
Smoking (current smokers)%	15.20	18.50	0.222	14.30	19.30	0.059
Alcohol consumption (portions/week)	1.42 ± 2.64	2.03 ± 3.29	0.004	1.40 ± 2.65	2.05 ± 3.27	0.002
Physical activity (hours/week)	2.26 ± 3.09	2.08 ± 3.15	0.417	2.34 ± 3.29	2.00 ± 2.94	0.132

Sugared beverages include: sparkling sugared beverage, fruit juice, carrot juice, sugared iced tea, flavored water, sugared iced coffee.
Statistical analysis performed using *t* test.
AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HbA1C, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; HRI, hepato-renal index; NAFLD, non-alcoholic fatty liver disease.
* HOMA-IR >3.31 (score).

Israeli population (OECD data: https://data.oecd.org/agroutput/meat-consumption.htm). High meat eaters were slightly younger, were more likely to be men, had a higher BMI and caloric intake, as well as a worse metabolic profile, including higher HOMA-IR, serum uric acid and lipids. The prevalence of NAFLD was higher among the high meat eaters, supported by higher mean levels of HRI, as well as higher alanine aminotransferase (ALT) and Gamma-glutamyl transferase (GGT) serum levels. High meat eaters did not have significantly worse lifestyle habits, with similar levels of physical activity, smoking and sugared drink consumption, but had slightly higher alcohol consumption within the range of adequate intake, and as expected, higher consumption of saturated fat and cholesterol which were adjusted for in the multivariate analysis as potential confounding or mediating factors (Table 1).

Multivariate analysis of the association between meat subtypes consumption and NAFLD or IR

High total meat intake (above the median) was independently associated with higher odds for both NAFLD (OR 1.49; 95% CI 1.05–2.13; $p = 0.028$) and IR (OR 1.63; 95% CI 1.12–2.37; $p = 0.011$), adjusting for potential confounders (Table 2). Similarly, high red and/or processed meat (joint categories) intake was independently associated with higher odds for both NAFLD (OR 1.47; 95% CI 1.04–2.09; $p = 0.031$) and IR (OR 1.55; 95% CI 1.07–2.23; $p = 0.020$), adjusting for potential confounders (Table 2). High processed meat intake did not remain significantly associated with IR or with NAFLD in the fully adjusted model (Table 2). Further adjustment of the association between high red and/or processed meat and NAFLD for type 2 diabetes did not change the results (OR 1.45; 95% CI 1.01–2.07; $p = 0.042$).

A dose-response association between red and/or processed meat consumption and NAFLD and IR was further demonstrated in both univariate and multivariate analysis (Fig. 2).

In addition, in a multivariate linear regression, including only non-diabetic participants (n = 672), high total meat intake (B = 0.07, $p < 0.001$), high red and/or processed meat (B = 0.05, $p = 0.023$) and high processed meat (B = 0.04, $p = 0.001$) intake were independently associated with log HOMA levels, adjusting for the same variables as in model B (Table 2).

Univariate and multivariate analysis of the association between consumption of calculated HCAs and meat prepared in unhealthy cooking methods and NAFLD or IR

The prevalence of NAFLD did not differ, while the prevalence of IR was higher among individuals with high (above the median) calculated HCA consumption (22.30% *vs.* 34.70%, $p = 0.010$) or higher consumption of meat cooked by unhealthy methods (22.20% *vs.* 36.00%, $p = 0.004$) (Fig. 3). Multivariate analysis confirmed the lack of association with NAFLD, and the strong positive association with IR for high HCA intake (OR 2.22; 95% CI 1.28–3.86; $p = 0.005$) and for high consumption of meat cooked by unhealthy methods (OR 1.92; 95% CI 1.12–3.30; $p = 0.018$) (Table 2).

Univariate and multivariate analysis of the association between meat subtypes consumption and IR among NAFLD diagnosed individuals

Of the 305 individuals with NAFLD, 167 (54.8%) had IR. The prevalence of IR was higher among those with high total meat consumption (59.2% *vs.* 47.9%, $p = 0.052$) or higher red and/or processed meat consumption (60.1% *vs.* 46.7%, $p = 0.021$). In the sub-sample of individuals who completed the meat questionnaire, of the 122 with NAFLD, 64 (52.5%) had IR. The prevalence of IR was higher among individuals with higher calculated HCA consumption (64.6% *vs.* 38.6%, $p = 0.004$) or higher consumption of meat cooked by unhealthy methods (68.3% *vs.* 37.1%, $p = 0.001$).

High red and/or processed meat (joint categories) intake was independently associated with higher odds of IR (OR 1.66; 95% CI 1.01–2.74; $p = 0.046$), adjusting for potential confounders (variables that differed between the compared groups) (Table 3). The multivariate analysis also showed a positive association between IR and high HCA intake (OR 4.38; 95% CI 1.82–10.57; $p = 0.001$), as well as high consumption of meat cooked by unhealthy methods (OR 4.17; 95% CI 1.79–9.71; $p = 0.001$) (Table 3).

Table 2. Multivariate analysis of the association between meat subtypes consumption and NAFLD or IR.

	NAFLD		IR	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Total meat (†portions/day) (n = 789)				
Model[a]				
<1.10	1 (ref)		1 (ref)	
≥1.10	1.52 (1.08–2.14)	0.017	1.65 (1.15–2.36)	0.007
Model[b]				
<1.10	1 (ref)		1 (ref)	
≥1.10	1.49 (1.05–2.13)	0.028	1.63 (1.12–2.37)	0.011
Red and/or processed meat (†portions/day) (n = 789)				
Model[a]				
<0.33	1 (ref)		1 (ref)	
≥0.33	1.46 (1.04–2.04)	0.029	1.51 (1.06–2.15)	0.022
Model[b]				
<0.33	1 (ref)		1 (ref)	
≥0.33	1.47 (1.04–2.09)	0.031	1.55 (1.07–2.23)	0.020
Processed meat (†portions/day) (n = 789)				
Model[a]				
<0.10	1 (ref)		1 (ref)	
≥0.10	1.21 (0.86–1.69)	0.272	1.41 (1.00–2.00)	0.053
Model[b]				
<0.10	1 (ref)		1 (ref)	
≥0.10	1.20 (0.85–1.68)	0.294	1.37 (0.96–1.96)	0.082
(N = 357)				
Total calculated HCAs (†ng/day) (n = 357)				
Model[a]				
<253	1 (ref)		1 (ref)	
≥253	1.23 (0.78–2.00)	0.403	2.06 (1.20–3.53)	0.009
Model[b]				
<253	1 (ref)		1 (ref)	
≥253	1.20 (0.73–1.96)	0.474	2.22 (1.28–3.86)	0.005
Meat cooked in unhealthy methods (†portions/week) (n = 357)				
Model[a]				
<1.00	1 (ref)		1 (ref)	
≥1.00	1.08 (0.66–1.74)	0.769	1.83 (1.08–3.09)	0.024
Model[b]				
<1.00	1 (ref)		1 (ref)	
≥1.00	1.06 (0.65–1.74)	0.823	1.92 (1.12–3.30)	0.018

Statistical tests performed using logistic regression.
HCAs, heterocyclic amines; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.
† Divided above and below the population's median consumption.
[a] Adjusted for: Age, gender, energy intake per day and BMI.
[b] Adjusted for all the above and: weekly hours of physical activity, smoking status, weekly alcohol portions, saturated fat (percent of daily energy) and cholesterol intake.

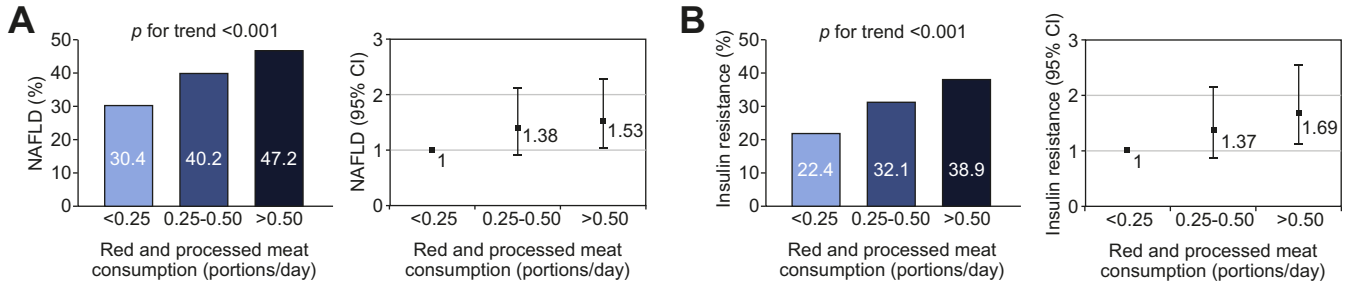

Fig. 2. Dose-response univariate and multivariate association between red and/or processed meat and NAFLD or IR. (A) Association between red and/or processed meat (portions/day) and NAFLD, univariate (left panel) and multivariate (right panel). (B) Association between red and/or processed meat (portions/day) and IR, univariate (left panel) and multivariate (right panel). Univariate *p* values are calculated by Chi-Square test, OR, adjusted for all variables listed in Table 2 model b, and 95% CI are calculated by logistic regression. In each category: <0.25, 0.25–0.5, >0.5, n = 330, 168, 288, respectively. IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.

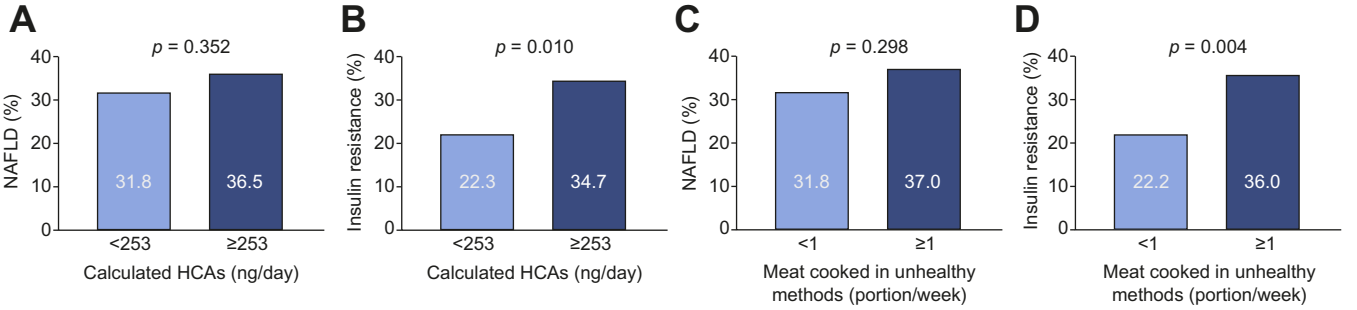

Fig. 3. The prevalence of NAFLD and IR by calculated HCAs or by meat cooked using unhealthy methods consumption. (A, C) Prevalence of NAFLD, and (B, D) prevalence of IR (HOMA >2.97), by calculated HCAs or by consumption of meat cooked using unhealthy methods (divided by median). *p* values are calculated by Chi-Square test. In each category below and above the median consumption: n = 195, 162 for unhealthy methods and n = 179, 178 for HCAs, respectively. HCAs, heterocyclic amines; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.

Table 3. Multivariate analysis[a] of the association between meat subtypes consumption and IR among NAFLD diagnosed individuals.

	IR	
	OR (95% CI)	*p* value
Food frequency questionnaire sample (n = 305)		
Total meat (†portions/day)		
<1.10	1 (ref)	
≥1.10	1.53 (0.92–2.53)	0.101
Red and/or processed meat (†portions/day)		
<0.33	1 (ref)	
≥0.33	1.66 (1.01–2.74)	0.046
Processed meat (†portions/day)		
<0.10	1 (ref)	
≥0.10	1.29 (0.79–2.11)	0.316
Meat questionnaire sub sample (n = 122)		
Total calculated HCAs (†ng/day)		
<253	1 (ref)	
≥253	4.38 (1.82–10.57)	0.001
Meat cooked in unhealthy methods (†portions/week)		
<1.00	1 (ref)	
≥1.00	4.17 (1.79–9.71)	0.001

Statistical tests performed using logistic regression.
NAFLD, non-alcoholic fatty liver disease; IR, insulin resistance; OR, odds ratio.
† Divided above and below the population's median consumption.
[a] Adjusted for: age, gender, BMI, daily energy intake and weekly alcohol consumption.

Discussion

World meat consumption has increased in the last decades,[36] while evidence of its harmful effect is mounting, particularly of red and processed meat consumption. Meat consumption has been linked to induction of impaired glucose and insulin homeostasis,[15] diabetes and cardiovascular disease (CVD).[16] In parallel, the prevalence of NAFLD is growing[1] and expected to grow further, leading to an exponential increase in disease burden.[41] This increased prevalence may be attributed, at least in part, to the adoption of Western diets high in processed and red meat, as suggested according to the results of the current study. We have demonstrated an independent positive association of high consumption of total, and specifically red and/or processed meat, with both NAFLD and IR. Furthermore, consumption of meat cooked by unhealthy methods was independently associated with HCAs and IR. Within the group of patients with NAFLD, similar positive associations of red and/or processed meat, meat cooked in unhealthy methods and HCAs were demonstrated with IR, which is a risk factor for disease severity.[42] Our results are in accordance with the few studies published on this topic, indicating an association between total meat consumption and NAFLD[20] and a potential protective role of a vegetarian diet[43] or a low animal protein diet.[44] In terms of food preparation methods, in a case-control study,[5] grilled meat or fish intake more than once a week increased the odds for NAFLD by about twofold.[5] Furthermore, indirect support for our findings stems from the protective effect of the Mediterranean diet in NAFLD,[45] advocating low red meat and especially processed meat intake, and actually defined as "primarily a plant-based diet".[46]

In contrast to NAFLD, the association between meat consumption and type 2 diabetes and CVD, which coexist and share common pathogenic pathways with NAFLD and IR,[47] was extensively studied. In a meta-analysis of prospective cohort studies,

processed meat consumption was associated with the risk of all-cause and CVD-related mortality,[48] and with 42% higher risk of CVD and 19% higher risk of diabetes per 50 g serving/day,[49] but no clear association was observed with unprocessed red meat. Conversely, in the Women's Health study, intake of both red and processed meat was associated with increased risk of type 2 diabetes.[50] Other cohort studies support these findings, mostly linking diabetes with the intake of processed red meat, and to a lesser extent and consistency with red meat in general.[51–53] In the current study, there was only an association between processed meat *per se* with IR, but not with NAFLD, perhaps because of a relatively low consumption in this study population.

The mechanisms by which meat intake is related to NAFLD are unknown. It can be claimed that the harmful association with meat may, at least partially, be related to a generally less healthy diet or lifestyle characterizing people who eat more red or processed meat, rather than a causal effect of meat. However, in the current study we meticulously adjusted the association with meat for other nutritional and lifestyle parameters to minimize confounding as much as possible. Indeed, there are several plausible mechanisms that can explain the observed associations, including a role for; SFA and cholesterol, AGEs, sodium, nitrates/nitrites and heme-iron, based on data from clinical trials and animal models.[8–12] Several epidemiological studies demonstrated a positive association between high SFA intake and risk of diabetes mellitus or IR.[54–56] In addition, intervention studies showed that a high SFA diet leads to reduced insulin sensitivity[57,58] and, in fact, SFA ingestion rapidly increases hepatic lipid storage and IR.[8] However, in this study, the full multivariate analysis adjusted for SFA and cholesterol intake, indicating a sustainable association of meat with NAFLD and IR, beyond the potential mediating effect of these dietary fats. Iron causes cellular oxidative stress which decreases insulin action,[59] and in a meta-analysis heme-iron intake was associated with 30% higher risk of diabetes.[60] High levels of sodium, that are about 400% higher in processed meats,[36] may also play a role since salt intake was suggested to be associated with increased risk of NAFLD.[13] Processed meat also contains an average of about 50% more nitrates than unprocessed red meat.[49] Nitrites and nitrates used in the preservation of processed meat are converted into nitrosamines, which are related to IR and diabetes in animal studies.[12]

Regarding the cooking method, exposure to dry heat and high temperature can generate high levels of both HCAs and AGEs in meat.[7,23,24,61] Because of the involvement of myoglobin in the formation of AGEs, red meat and particularly processed red meat have the highest AGEs content.[12] Indeed, when compared to simple steatosis and controls, serum AGEs have been demonstrated to be related with non-alcoholic steatohepatitis (NASH), and to positively correlate with IR and negatively with adiponectin.[14] In addition, lowering dietary AGEs by mild steam cooking instead of high-temperature cooking lowers IR in healthy people.[61] In contrast to AGEs, the role of HCAs in NAFLD formation was never tested and should be explored. Potential mechanisms for NAFLD may be related to the formation of reactive species during HCA metabolism, which can cause oxidation of lipids, proteins and nucleic acids, resulting in oxidative stress, cell damage and loss of biological function.[27] HCAs were also demonstrated to be bioactive in adipocytes *in vitro*, leading to increased expression of genes related to inflammation, diabetes and cancer risk.[62]

In view of the evidence-based harmful effect of red and processed meat, the dietary guidelines for cardiometabolic health limit the recommended intake of unprocessed red meats to no more than 1–2 servings/week of 100 g, and of processed meats to no more than 1 serving/week of 50 g.[16] In agreement with these recommendations, in our study, one portion of meat translates to about 100 g, meaning that a weekly consumption of more than two servings (of 100 g) of red and/or processed meat is associated with NAFLD and IR and a weekly consumption of more than one portion of processed meat (of 50 g) tends to be associated with IR. Although the specific effect of different types of meat and their quantities in NAFLD requires further research, these recommendations may be helpful in the treatment of patients with NAFLD at least in terms of CVD and diabetes prevention, and maybe for NAFLD prevention by reducing IR.

This study has several limitations. Firstly, the cross-sectional design of the study does not allow causal inference. Secondly, meat consumption was self-reported and thus prone to reporting bias. However, since the participants and the research team were blinded to the AUS and blood tests results, it is a non-differential reporting bias and therefore it may have only weakened the observed associations. Thirdly, NAFLD was determined non-invasively as appropriate for a general population study. However, ultrasonography, in contrast to liver biopsy, did not enable us to study the association between meat consumption and NASH. With regard to NAFLD detection, AUS was performed in all individuals using the same equipment and by the same experienced radiologist across all levels of meat consumption, meaning that this potential non-differential bias can only weaken the observed associations. Lastly, nutritional data has been collected within a single country, which may impact on the generalizability across populations with differing dietary patterns.

In conclusion, high meat consumption in general, and specifically high red and processed meat consumption, are independently associated with NAFLD and IR, regardless of saturated fat intake. In addition, high consumption of meat cooked by unhealthy methods and high HCA intake are independently associated with IR and thus may have a role in the pathogenesis of NAFLD.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Zelber-Sagi Shira; conceived and designed the study, supervised on data collection and analysis and wrote the manuscript, Ivancovsky-Wajcman Dana; designed the study, did the data collection and analysis and wrote the manuscript, Fliss Isakov

Naomi; designed the study and did the data collection, Webb Muriel; performed the ultrasonography evaluation and critically reviewed the manuscript, Orenstein Dana; data collection and quality control, Shibolet Oren; critically reviewed the manuscript, Kariv Revital; conceived and designed the study, supervised on data collection and critically reviewed the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2018.01.015.

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