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Article

The Association between Self-Reported Daily Energy Intake from Carbohydrates with Insulin Resistance in Healthy Weight Nor-Mal-Weight Individuals

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Abstract: Carbohydrates form the major source of energy in Asian diets. A lower carbohydrate diet became the recommended golden standard for healthy lifestyle. However, the effects of low-carbohydrates diet on health in apparently healthy individuals have been poorly studied, especially in relation to insulin resistance syndrome (IRS). A total of 120 healthy weight participants with no previous history of a major medical condition and an average BMI of ≤ 25kg/m2 were recruited. Self-reported dietary intake and objective physical activity by accelerometry were tracked for seven days. Participants were divided into three categories according to their dietary intake of carbohydrates. Blood samples were collected for metabolic markers analysis. HOMA of insulin resistance (HOMA-IR), β-cell function (HOMA-B) and C-peptide were used to evaluate glucose homeostasis. The consumption of low carbohydrates (less than 45% of total energy) significantly correlated with higher HOMA-IR, Lower HOMA-β (%) compared to moderate carbohydrate intake (between 45% to 65%). However, only the HOMA-β (%) was significantly influenced by carbohydrates intake. Moreover, low carbohydrates intake was significantly associated with elevated C-peptide secretion. The substitution of carbohydrates with other macronutrients, such as fat and proteins in the Atkins/ketogenic diet, resulted in a pronounced induction of IRS-related inflammatory markers; FGF2, IP-10, IL-6, IL-17A, MDC and reduction of IL-13. Overall, the presented data highlight, for the first time, that low carbohydrate intake results in significant glucose homeostasis imbalance that may be driven by a heightened state of inflammatory response.

Keywords: Carbohydrates; keto diet; IRS 3; C-peptide

1. Introduction

Insulin resistance syndrome (IRS) is a modern-day epidemic. With the increase of research endeavors and focus on IRS, it has become evident that IRS can drive the disease pathogenesis of several clinical syndromes such as type 2 diabetes, cardiovascular disease, essential hypertension, and nonalcoholic fatty liver disease (NAFLD) **[1, 2]**. Both hyperinsulinemia and β-Cell dysfunction are involved in the development of IRS **[3]**. Even though

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the IRS can be triggered by several factors, such as genetics and age, obesity and dietary intake have been considered as the major modulator of IRS. To this end, maintaining a normal weight became the recommended prevention strategy. The current recommended dietary guideline for treating obesity revolves around reducing daily energy intake, portion control, and improving diet quality to achieve a calorie deficit status **[4]**. However, over the past decade, further research unraveled the benefits of re-directing the weight loss strategy toward maintaining a particular level of micronutrients within the daily dietary intake. With carbohydrates being the macronutrient with the most significant impact on postprandial blood glucose response, low carbohydrate diets, such as Atkins and keto diets, became the gold standard for weight loss **[5, 6]**. It is believed that during low carbohydrate intake, the body undergoes ketogenesis, a process that switches the utilization of glucose from the carbohydrate as the energy source to the use of ketone bodies in mitochondria for ATP synthesis, shifting the metabolic process to ketosis favoring pathways **[7]**. This metabolic shift following low-carb dietary interventions is a cornerstone of the weight loss mechanism. On the other hand, overproduction of ketone bodies from increased ketogenesis can trigger metabolic acidosis **[8, 9]**. The influence of low carbohydrates diet and the activation of the ketogenesis pathway in individuals with obesity, especially those with metabolic complications such as type 2 diabetes, has been proven effective in reducing body weight **[6]**; however, the short or long-term significance of this lifestyle in normal-weight individuals remains scientifically unclear. Albeit, widely publicized by social media as being universally a healthy option.

This study aims to explore the effect of low carbohydrate intake in individuals with a BMI of ≤ 25 kg/m2. Through dietary self-reporting, we identified the percentage of daily energy intake from carbohydrate for each individual. Using the recommended National Standard, we divided the 120 participants into following three groups: (i) low carbohydrate (LC) group comprising of those consuming less than 45% of daily energy percentage from carbohydrate), (ii) recommended range of carbohydrate (RC) group comprising of those consuming 45-65% of daily energy percentage from carbohydrate, and (iii) high carbohydrate (HC) group comprising of those consuming higher than 65% of daily energy percentage from carbohydrate. We found that low carbohydrate intake (less than 45% of total energy) was associated with a higher HOMA-IR as well as a lower HOMA-β (%), as compared to moderate carbohydrate intake (45% to 65 %). The data show that carbohydrate consumption had a substantial impact on HOMA-β (%) assessment. Interestingly, low carbohydrate consumption associated with increased C-peptide levels, which correlated positively with IRS-related inflammatory markers including FGF2, IP-10, IL-6, IL-17A, and MDC, however, a negative association was found with the pluripotent hematopoietic factor IL-3. Overall, the findings show for the first time that reduced carbohydrate intake in normal weight individuals may cause defective glucose homeostasis driven by C-peptide elevation.

2. Results

2.1 Cohort characteristics

The general characteristics of the study participants, dietary and energy intake data are summarized in **table 1**. Based on WHO chart for age and sex, all participants were within the normal range of BMI, with average BMI of 22.7 ± 2.4 kg/m2. In our study, 63% of participants were females and mean age of all participants was 32.2 ± 5.7. The mean systolic and diastolic blood pressure measurements were normal (109.9 ± 11.3 and 67.15 ± 9.8, respectively) and the average heart rate (HR) was 71.4 ± 10.9. According to the general guidelines set forth by Kuwait ministry of health (MOH), all participants showed normal blood lipid profile. The study participants had normal levels of serum triglycerides (TG) (0.87 ± 0.38 mmol/L), total cholesterol (4.6 ± 0.8 mmol/L), and HDL-C (1.49 ± 0.34). All participants also showed normal glucose homeostasis, with the average fasting glucose of

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4.9 ± 0.64 mmol/L and serum insulin level of 3.7 ± 2.11 U/ml. All individuals were within the normal range of HOMA-β (%) (74.8 ± 58.9) and HOMA-IR (< 2.5) indices. The participants also had normal fasting blood C-peptide levels (1.4 ± 0.37 ng/mL). The average total calorie intake per day for all participants was 2143.8 ± 571.9 kcal, with most energy consumed from carbohydrate 49.5 ± 12.5% of daily energy.

Table 1: Physical characteristics, dietary and energy intake

Physical characteristics of subjects	Mean ± SD Male/female (57/63)	Physical characteristics of subjects	Mean ± SD Male/female (57/63)
Age (years)	32.2 ± 5.7	HDL cholesterol (mmol/l)	1.49 ± 0.34
Weight (kg)	65.0 ± 12.1	Insulin Con. (mu/l)	3.7 ± 2.11
Height (cm)	168.3 ± 11.8	HOMA-IR	0.82 ± 0.48
BMI (kg/m2)	22.7 ± 2.4	HOMA-β%	74.8 ± 58.9
Waist circumference(inch)	29.4 ± 3.6	C-Peptide	1.4 ± 0.37
Hip circumference (inch)	40.3 ± 9.8	Total Calories (kcal)	2143.8 ± 571.9
Fat weight (kg)	23.3 ± 11.9	Carbs (g)	264.6 ± 95.8
lean weight (kg)	45.7 ± 9.7	Carb energy %	49.5 ± 12.5
fat %	25.7 ± 9.3	Fat (g)	56.7 ± 28.0
BP/ systolic (mmHg)	109.9 ± 11.3	Fat energy %	19.2 ± 10.3
BP/diastolic (mmHg)	67.15± 9.8	Protein (g)	91.2 ± 48.8
HR	71.4 ± 10.9	Protein energy %	17.8 ± 10.1
		Chol (mg)	197.2 ± 138.1
Fasting glucose (mmol/l)	4.9 ± 0.64	Sodium (mg)	1375.8 ± 397.7
Triglycerides (mmol/l)	0.87 ± 0.38	Sugars (g)	89.5 ± 76.8
Total cholesterol (mmol/l)	4.6 ± 0.8	Fiber (g)	22.6 ± 16.7

2.2 Association of the percentage of energy intake from dietary carbohydrate with insulin resistance

As per international health guidelines, the recommended daily energy intake from carbohydrate is set between 45 to 65% of daily calorie intake [10, 11]. Based on these criteria, study participants were divided into three tertile (Q1, Q2, Q3) groups as: low carbohydrate (LC/Q1) group (> 45% of daily energy intake from carbohydrate); recommended range of carbohydrate (RC/Q2) group (those consuming 45- 65% of daily energy intake from carbohydrate); and high carbohydrate (HC/Q3) group (< 65% of daily energy intake from carbohydrate). Group characteristics are summarized in **Table 2**, which shows significant inter-tertile differences with regard to lean weight (Q1 vs Q2 and Q3), HOMA-β% (Q1 vs Q2), fasting glucose, insulin, C-peptide, and HOMA-IR (Q1 vs Q2 and Q2 vs Q3). While, no significant differences were found regarding anthropometric characteristics, lipid profile, and total calorie intake per day.

We also found no significant differences in the level of objectively measured physical activity, as indicated below in **table 3**.

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Table 2: Inter-tertile differences between groups based on daily calorie intake (%) from carbohydrate

Physical characteristics of subjects	<45%(Q1) (N= 38)	45-65%(Q2) (N=62)	>65% (Q3) (N=20)	p-vale
Age (years)	32.1 ± 4.9	31.9 ± 4.7	33.3 ± 6.8	0.5512
Weight (kg)	64.1 ± 10.1	66.1 ± 12.8	63.0 ± 13.7	0.5383
Height (cm)	167.8 ± 11.4	170.1 ± 11.6	163.4 ± 12.5	0.0895
BMI (kg/m2)	22.7 ± 2.9	22.9 ± 2.4	23.2 ± 2.6	0.7960
Waist circumference(inch)	29.9 ± 3.6	31.7 ± 3.1	31.3 ± 5.4	0.0794
Hip circumference (inch)	41.4 ± 12.1	39.6 ± 4.0	39.6 ± 3.8	0.4826
Fat weight (kg)	20.3 ± 11.2	23.4 ± 12.1	27.8 ± 11.6	0.0744
lean weight (kg)	49.5 ± 11.6	43.4 ± 11.4	42.2 ± 9.3	**0.0075 ψ Φ**
fat %	21.3 ± 10.3	25.9± 10.0	26.8 ± 7.3	0.0922
Total calorie intake (Kcal)	2104.7 ± 581	2124 ± 588	2277 ± 504	0.5141
BP/ systolic (mmHg)	110.6 ± 11.7	109.3 ± 11.1	110.9 ± 11.6	0.8000
BP/diastolic (mmHg)	67.7 ± 10.4	66.5 ± 10.2	67.8 ± 7.1	0.8095
Heart Rate	70.8 ± 11.7	70.8 ± 10.7	74.7 ± 9.4	0.3814
Fasting glucose (mmol/l)	5.2 ± 0.7	4.7 ± 0.5	5.5 ± 0.5	**0.0011ψΔ**
Triglycerides (mmol/l)	0.88 ± 0.3	0.86 ± 0.4	0.89 ± 0.3	0.9361
Total cholesterol (mmol/l)	4.5 ± 0.7	4.6 ± 0.7	4.9 ± 1.0	0.2056
HDL cholesterol (mmol/l)	1.5 ± 0.32	1.45 ± 0.37	1.6 ± 0.3	0.1778
Insulin Con. (mU/l)	4.5 ± 1.4	3.2 ± 2.3	4.6 ± 2.2	**0.0022 ψΔ**
HOMA-IR	1.05 ± 0.37	0.70 ± 0.47	1.0± 0.55	**0.0034 ψΔ**
HOMA-β	56.3 ± 30.3	80.5 ± 59.4	58.9 ± 37.0	**0.0324 ψ**
C-Peptide (pg/ml)	1.5 ± 0.36	1.3 ± 0.34	1.56 ± 0.36	**0.0065 ψΔ**

Results are presented as mean ± SEM, Φ Q1 vs Q3, Ψ Q1 vs Q2, Δ Q2 vs Q3

Table 3: Comparison of physical activity levels across tertile groups

Physical Activity level	>45%(Q1) (N= 38)	45-65%(Q2) (N=62)	<65% (Q3) (N=20)	p-vale
Overall activity (%)	29.2 ± 6.2	30.3 ± 7.5	32.4 ± 6.3	0.2436
Light intensity (%)	22.5 ± 4.5	23.9 ± 5.2	25.4 ± 5.8	0.1066
Moderate intensity (%)	5.6 ± 2.3	5.9 ± 2.4	5.7 ± 1.2	0.8067

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Vigorous intensity (%)	1.04 ± 0.9	0.89 ± 0.7	1.1 ± 0.69	0.3724
Average MET rate/day	1.54 ± 0.13	1.57 ± 0.21	1.59 ± 0.12	0.3500
Average step count/day	9541 ± 3608	11781 ± 17031	10134 ± 2174	0.6662

However, individuals consuming RC were found to have significantly lower HOMA-IR when compared to those consuming LC ($p \leq 0.05$) and HC ($p \leq 0.01$) and higher HOMA-β (%) (**Figure 1A and B**). It was also observed that participants consuming RC had significantly lower C peptides in their serum compared to both LC and HC groups ($p < 0.05$) (**Figure 1C**).

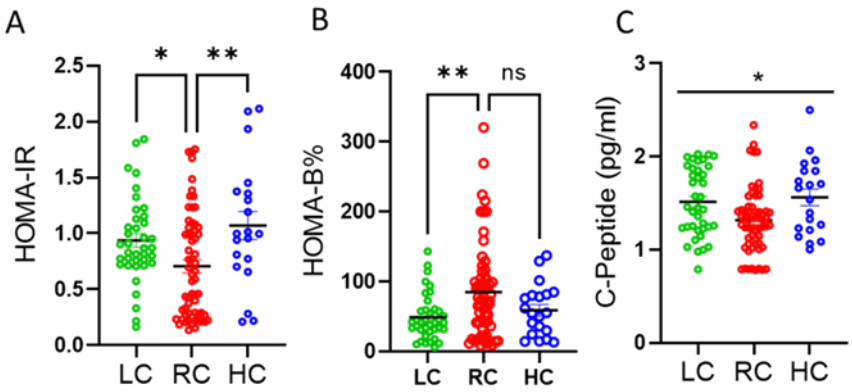

Figure 1 **The effect of different levels of carbohydrate intake on glucose homeostasis**. Self-reported dietary logs from 120 participants were analyzed and divided three categories according to their carbohydrate intake. Low carbohydrate (LC) (those consuming less than 45% of daily energy percentage), recommended range of carbohydrate (RC) (those consuming less than 45- 65% of daily energy percentage) and high carbohydrate (HC) (those consuming higher than 65% of daily percentage). (A) HOMA-IR homeostasis model assessment of insulin resistance, (B) HOMA-beta homeostasis model assessment of β-cell function (C) C-peptide section. Data are expressed as mean ± SD. Data analyzed by one-way ANOVA with Tukey's multiple comparisons test. ns = non-significant, *$p \leq 0.05$ and **$p \leq 0.01$.

To further investigate these finding, a Pearson's correlation analysis was preformed to determine the associations between carb energy % and surrogate markers of insulin resistance; β-cell function, and insulin secretion (HOMA-IR, HOMA-β% and C peptide levels, respectively). No significant association was found between HOMA-IR and carb energy % across all groups (**Figure 2A**). However, unlike RC and HC groups, a clear trend of negative HOMA-IR association with carbohydrate energy % was observed in LC group. Interestingly, fasting serum C-peptide levels in LC group had negative association with carbohydrate energy %, while C-peptide levels in RC and HC groups tended to have a direct association with carbohydrate energy % (**Figure 2B**). In terms of β-cell function assessment, HOMA-%B index associated positively with levels of carbohydrate energy % in both LC and RC groups, whereas HOMA-β% tended to have a negative association with carbohydrate energy % in HC group (**Figure 2C**).All in all, these data clearly indicate that low carbohydrate intake might be associated with insulin resistance and that the consumption of 45 to 65% of energy intake from carbohydrate is important to maintain normal glucose hemostasis in normal weight individuals.

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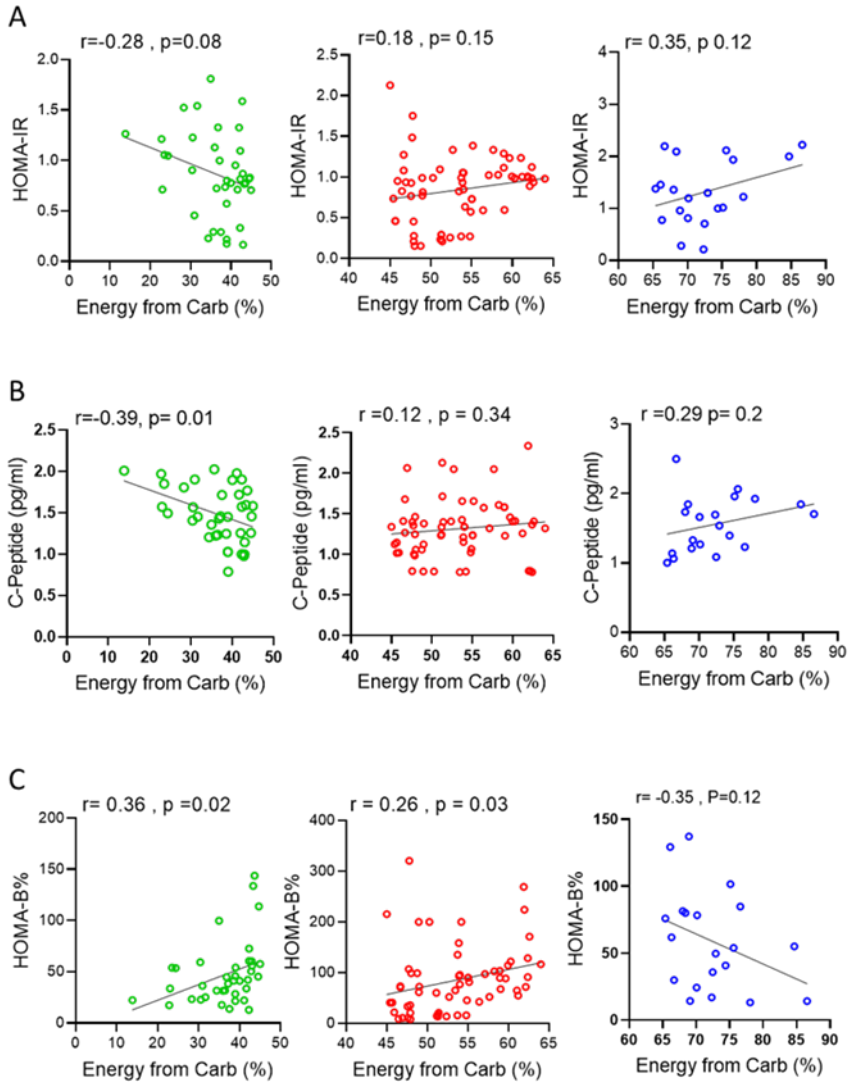

Figure 2 Low carbohydrate intake is associated with trends in dysfunctional glucose homeostasis. Pearson's correlation coefficient analysis was conducted to investigate the association between (A) HOMA-IR, (B) C-peptide secretion and (C) HOMA-β% and the level of carbohydrate intake % of energy in all three groups. All data are expressed as mean ± SD. P≤0.05 was considered statistically significant

2.3 Association of the C-peptide levels with circulatory inflammatory markers

C-peptide is a biologically active short polypeptide (31 amino acids) that serve as a diagnostic biomarker to distinguish between type 1 and type 2 diabetes and a strong indicator of insulin biosynthesis and insulin resistance syndrome (IRS) (13-15). During the past decade, several studies have demonstrated a biological effect of plasma circulating C-peptide on activating inflammatory signaling pathway (16, 17). To this end, we questioned the possible association of elevated level of C-peptide under low carbohydrate intake with insulin resistance related inflammatory cytokines. Multiplex cytokine assay was conducted to investigate the secretion of these cytokines known to be involved in several metabolic disorders such as diabetes and insulin resistance syndrome. Out of the 41 inflammatory mediators investigated, only seven (IP-10 ; p=0.045, VEGF ; p=0.049 , IL-6; 0.049, IL-17A ; p=< 0.0001, FGF-2 ; p= 0.025, MDC; p=0.019 and GRO; p= 0.035) were found to be significantly elevated in LC group when compared with RC group and only one

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cytokine (IL-3 ; p=0.036) was found to be significantly reduced in LC group compared to HC group, as depicted in **Table 4**.

Table : 4 Inter-tertile group comparison of plasma inflammatory markers

Pearson's correlation analysis further showed that out of those eight cytokines/bioactive factors, five were found to be positively associated with C-peptide expression (FGF-2; r = 0.48, p = 0.002, IP-10; r = 0.39 , p = 0.014, IL-6; r = 0.38, p = 0.016, IL-17A; r = 0.45, p = 0.003 and MDC; r = 0.32, p = 0.04) (**Figure 3A-E**), one was negatively associated with C-peptide expression (IL-3; r = 0.38, p = 0.01) (**Figure 3F**), and two (VEGF and GRO) had no association with C-peptide levels (**Figure. 3G-H**).

Together, the presented data suggest that under the condition of low dietary carbohydrate intake, an association is found between the plasma C-peptide levels and IRS-related cytokines / mediators' expression, supporting the active role of C-peptide as a bioactive molecule and significance as an IRS biomarker.

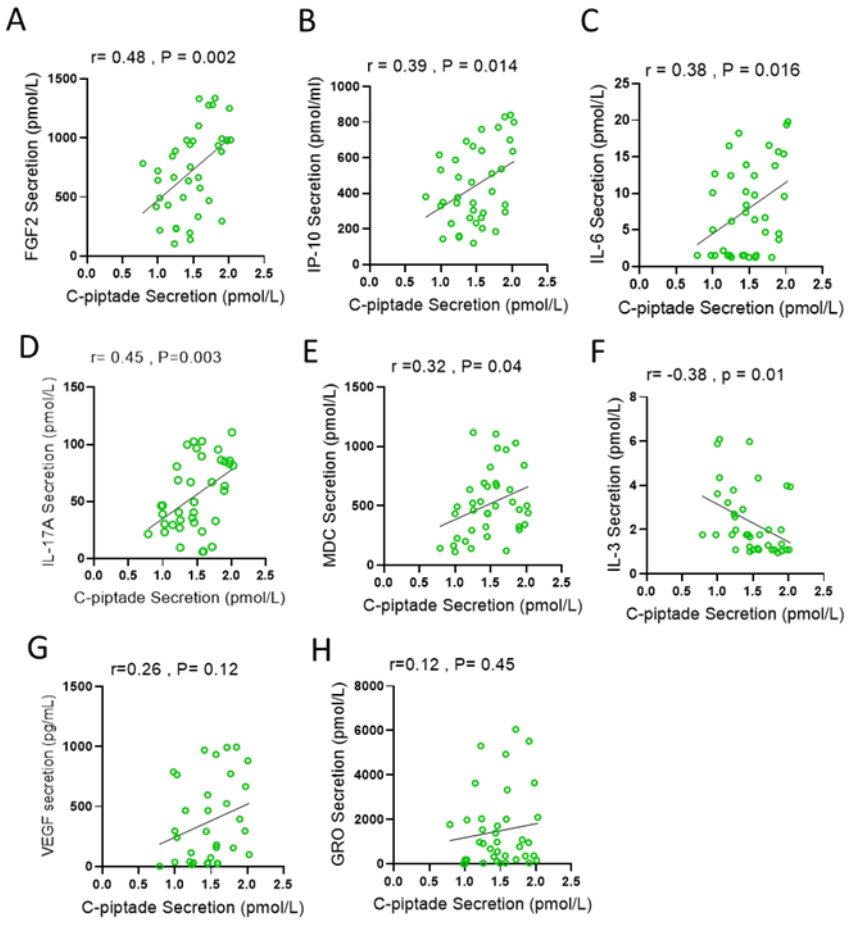

Figure 3 Low carbohydrate intake is associated with IRS-related inflammatory markers under low carbohydrate intake. Plasma levels of IRS related secretion of 41 cytokine and chemokine were

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Plasma Inflammatory markers	<45%(Q1) (N= 38)	45-65%(Q2) (N=62)	>65% (Q3) (N=20)	p-vale
IL-13 (pmol/L)	36.0 ± 98.2	18.8 ± 65.8	37.3 ± 44.3	0.5856
IP-10 (pmol/L)	441.9 ± 215.6	375.2 ± 283.5	413.6 ± 241.4	**0.0450** Ψ
IL-15 (pmol/L)	4.77 ± 10.9	3.7 ± 4.6	4.1 ± 4.3	0.7689
IL-9 (pmol/L)	240 ± 382.7	244.9 ± 342.0	155.5 ± 250.1	0.7209
MCP-1 (pmol/L)	318.7 ± 218.4	391.1 ± 231.0	272.6 ± 193.8	0.7378
IL-12P40 (pmol/L)	5.2 ± 6.99	7.22 ± 13.5	21.2 ± 66.0	0.3510
VEGF (pmol/L)	398.8± 340.5	224.5 ± 250.6	438.5 ± 442.1	**0.0497** Ψ
IL-12P70 (pmol/L)	105.1 ± 241.0	65.6 ± 170.9	37.7 ± 90.7	0.6026
IL-6 (pmol/L)	7.8 ± 6.1	5.5 ± 5.1	6.2 ± 7.3	**0.0495** Ψ
IL-1α (pmol/L)	50.1 ± 90.4	51.8 ± 123.1	72.4 ± 107.8	0.8394
IL-1RA (pmol/L)	134.1 ± 228.5	118.2 ± 170.5	101.9 ± 114.0	0.8933
IL-1β (pmol/L)	6.9 ± 6.2	7.8 ± 11.6	7.1 ± 8.3	0.9018
IL-8 (pmol/L)	24.6 ± 27.2	16.8 ± 19.0	15.0 ± 21.0	0.1686
Eotoxin (pmol/L)	112.3 ± 110.5	101.0 ± 105.2	68.6 ± 69.1	0.5115
IL-17A (pmol/L)	55.6 ± 30.6	27.4 ± 21.2	48.5 ± 29.2	**<0.0001** ΨΔ
MCP-3 (pmol/L)	17.6 ± 20.7	26.4 ± 74.3	10.2 ± 26.8	0.6758
sCSD40L (pmol/L)	2286.0 ± 3223.5	2419.0 ± 3347.7	2559.3 ± 2898.6	0.9745
G-CSF (pmol/L)	126.2 ± 205.6	239.6 ± 210.2	71.7 ± 118.6	0.4737
TNF-β (pmol/L)	13.5 ± 12.1	11.1 ± 11.2	11.7 ± 11.1	0.7739
GM-CSF (pmol/L)	13.9 ± 29.8	17.9 ± 24.8	25.9 ± 62.6	0.6782
Fractalkine (pmol/L)	244.4 ± 695.7	144.0 ± 238.6	206.4 ± 624.6	0.7785
TNF-α (pmol/L)	71.7 ± 74.7	64.8 ± 82.9	69.1 ± 26.4	0.9011
TGF-α (pmol/L)	44.1 ± 79.9	35.3 ± 57.9	12.6 ± 20.7	0.3683
Fit-3L (pmol/L)	13.9 ± 24.7	8.3 ± 19.7	13.6 ± 38.5	0.7164
FGF-2 (pmol/L)	723.2 ± 350.8	494.0 ± 425.4	457.3 ± 365.8	**0.0251** Ψ
IFN-γ (pmol/L)	1196.5 ± 2417.1	1054.3 ± 1857.7	1059.3 ± 1700.3	0.9698
IL-10 (pmol/L)	29.3 ± 56.4	41.5 ± 56.3	28.9 ± 42.8	0.6795
MDC (pmol/L)	514.2 ± 285.6	489.5 ± 436.9	546.1 ± 482.7	**0.0191**Ψ
GRO (pmol/L)	1282.5 ± 1687.8	1051.4 ± 2279.2	1530.1 ± 1930.6	**0.0352**Ψ
MIP-1β (pmol/L)	43.7 ± 54.5	32.2 ± 34.4	40.9 ± 47.7	0.5376
IFN-α2 (pmol/L)	112.6 ± 119.4	143.3 ± 173.7	169.7 ± 179.6	0.6369
MIP-1α (pmol/L)	25.8 ± 24.2	22.6 ± 20.3	25.0 ± 21.7	0.7654
IL-3 (pmol/L)	2.3 ± 1.5	9.2 ± 13.5	2.9 ± 4.7	**0.0367** Ψ
EGF (pmol/L)	211.5 ± 229.7	139.7 ± 156.0	95.0 ± 102.9	0.1984
IL-5 (pmol/L)	13.6 ± 29.6	7.5 ± 15.9	12.9 ± 13.9	0.7428
IL-2 (pmol/L)	2.2 ± 1.0	2.9 ± 2.0	6.8 ± 6.9	0.0871
IL-4 (pmol/L)	1.2 ± 2.4	2.3 ± 1.9	1.1 ± 2.3	0.1492

determined by multiplex Assay. (A-H) Pearson's correlation coefficient was conducted to investigate the relationship between significantly elevated cytokines in LC group compared to RC. All data are expressed as mean ± SD. P≤0.05 was considered statistically significant.

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3. Discussion

At present, low carbohydrate diets that are known to modulate macronutrient distribution have become much popular among the general population. Such diets are mainly consumed with the intentions to lose body weight and to improve metabolic profile in obesity and associated comorbidities, such as diabetes, non-alcoholic fatty liver disease, metabolic syndrome, and others. With the global rise of obesity and type 2 diabetes in humans, various dietary strategies that target restriction of calorie intake have been used widely as a common weight loss approach [12]. During the past decade, low carbohydrate diet has centered on weight loss in the obese and overweight individuals as well as in patients with or at risk for IRS, such as diabetes and atherosclerosis [13, 14]. Even though, the impact of low carbohydrate diet, especially the ketogenic diet, is found to be very effective in rapid induction of weight loss in both obese and overweight individuals, the impact of such diet remains to be well characterized in the normal weight or lean counterparts.

In this study, we have investigated for the first time to our knowledge, the effect of different dietary carbohydrate intake levels on the glucose hemostasis and IRS-related inflammatory markers in 120 normal weight individuals (BMI ≤25 kg/m²). Both IRS and insulin secretion competency were assessed using HOMA-based indices which have been duly validated against insulin clamp studies already [15, 16]. The data presented herein show that the individuals with low carbohydrate intake i.e., those consuming ≤45% of their daily calorie intake from carbohydrates presented with the trends of IRS. We found that under the condition of low carbohydrate intake, plasma glucose levels and consequently the HOMA-IR were both significantly elevated compared to weight-matched counterparts that consumed sufficient levels of carbohydrate for energy (45-56% of daily calorie intake); while the HOMA index representing beta-cell function (HOMA-β%) was found to be decreased under low carbohydrate intake diet. Increased plasma glucose levels and HOMA-IR values in normal weight individuals with low dietary carbohydrate intake that we observed is particularly interesting, and it may be explained by reasoning that proteolytic and lipolytic responses are enhanced under low dietary carbohydrate intake as part of alternate compensatory mechanisms to generate glucose from amino acids and glycerol, respectively [17]. Such gluconeogenic responses following a carbohydrate-restricted diet could be helpful for sustaining glycemia in healthy individuals, albeit, exacerbated glucose production and ketogenesis remain as the major concerns involved [18]. Carbohydrate restriction to very low levels may also have deleterious effects on intestinal homeostasis and fiber-derived antioxidant phenolic acids compared with a moderate or high carbohydrate intake [19]. Furthermore, a relative increase of ketone concentrations under low dietary carbohydrate intake may at first stimulate the pancreas to increase insulin release which may accumulate metabolic stress over time [20]. Besides, carbohydrate restriction induces lipolysis, releases free fatty acids, and increases citric acid cycle flux, all of which are known to promote reactive oxygen species (ROS) production [21] and suppress the function of beta cells [22], which could possibly explain the lower HOMA-β% values that we observed in normal weight individuals on low dietary carbohydrate intake.

Interestingly, plasma C-peptide levels were also found to be significantly elevated under low carbohydrate intake. A significant association was between the glucose homeostasis markers and low dietary carbohydrate intake, further supporting the effect of low-carbohydrate diet intake on glucose homeostasis. Carbohydrate metabolism is a fundamental biochemical process that ensures a constant supply of energy to living cells. Under prolonged consumption of low carbohydrate intake, the liver starts to produce ketone bodies as an alternative source of energy. Ketone bodies will travel from the liver to extrahepatic tissues to provide energy to different organs through breaking down of fatty acids and ketogenic amino acids [23, 24]. Even though ketone bodies are a very proficient

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respiratory fuel in comparison to glucose [25], the ketogenic diet induces hormonal changes that include the activation of phosphoenolpyruvate carboxykinase (PEPCK) [26], fructose 1,6-biphosphatase (FBPase) [27], and glucose 6-phosphatase (G6Pase) while inhibiting pyruvate kinase, 6-phosphofructo-1-kinase (PFK-1), and glucokinase (GCK) [28, 29], all of which are known to favor gluconeogenesis.

In our study population, we found that most individuals consuming low carbohydrate diet substituted their energy intake with higher protein and fat content (**Supplementary Figure S1**). To this end, we believe that this shift to high protein and fat content might be responsible, in part, for the increased plasma C-peptide levels in these individuals. In fact, the dietary protein was found to have an insulinotropic effect and promoted insulin secretion. Nonetheless, several studies suggested that a high dietary protein intake had an association with increased risk of type 2 diabetes and cancer [30-32].

Supplemental Figure. S1 Assessment of total energy from dietary intake divided by dietary source

Interestingly, a 20 year follow-up study by Fung et al. revealed that increased dietary intake of, but not limited to, meat, fish, and high-fat dairy products associated positively with higher plasma C-peptide levels, and directly associated with risk of colorectal cancer [33]. Indeed, in our study we have seen a significant association between lower carbohydrate intake and higher plasma C-peptide. Multiplex analysis of several inflammatory cytokines further revealed that plasma C-peptide upregulation associated positively with plasma FGF2, IP-10, IL-6, MDC and IL-17A levels. Notably, these cytokines have been previously identified to induce the development of insulin resistance and cause pathogenesis of type 2 diabetes [34-38]. On the other hand, a negative association was seen between the C-peptide levels and expression of anti-inflammatory cytokine IL-3, a pleiotropic regulator of inflammation [39].

Nevertheless, the present study is limited by certain caveats. For instance, the dietary intake in this study was assessed through self-reported diary logs. Even though adequate training was given to each participant along with a food scale, false reporting may not still be ruled out. We also have no record of how long each individual would have maintained this dietary lifestyle beyond the 7 days follow-up period. Therefore, the effects of long-term vs short-term dietary interventions of carbohydrate intake may not be evaluated.

4. Materials and Methods

4.1 Anthropometric, clinical, and dietary characteristics of the study participants

A total of 138 healthy adults with no past or current medical disorders, and BMI ≤ 25 kg/m2 were recruited in this study. All participants were informed about the study and the risks involved and asked to fill out the informed consent form and a health screen. Out of these, a total of 120 participants (57 male and 63 female), with a mean age of 31.9 ± 5.7 years, were found to be eligible for the final analysis. The study was approved by the Kuwait Ministry of Health (MOH) Ethics Board (2017/542). The exclusion criteria were; physical diagnosed diabetes, hypertension (>160/90 mmHg), anti-hypertensive therapy, a previous history of established coronary heart disease e.g. myocardial infarction, coronary artery bypass graft surgery, coronary angioplasty, a family history of early cardiac death (<40 years), history of cancer within the past two years, diagnosed depression, and the use of medications that could influence body weight due to effects on lipid or carbohydrate metabolism, also excluding pregnant or lactating females. None of the participants had

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physical disabilities that would prevent or severely limit physical mobility or physical activity. The characteristics of male and female participants are summarized below in **table 5**.

Table 5: Physical characteristics of study participants

Parameter	Males (N= 57)	Females (N=63)	p-vale
Age (years)	31.9 ± 5.2	32.5 ± 5.0	**0.5262**
Weight (kg)	69.4 ± 12.6	60.5 ± 10.0	**<0.0001**
Height (cm)	176.3 ± 9.3	160.3 ± 8.2	**<0.0001**
BMI (kg/m2)	22.7 ± 2.4	23.1 ± 2.7	0.4104
Waist circumference(inch)	31.7 ± 3.7	29.9 ± 3.8	**0.0148**
Hip circumference (inch)	40.3 ± 9.8	40.1 ± 4.1	0.8589
Fat weight (kg)	21.1 ± 11.5	25.0 ± 9.5	**0.0246**
Lean weight (kg)	57.9 ± 9.4	43.2 ± 9.1	**<0.0001**
Body fat (%)	22.2 ± 9.3	27.7 ± 5.7	**<0.0001**
BP/ systolic (mmHg)	111.9 ± 11.4	108 ± 11	**0.0596**
BP/diastolic (mmHg)	68.9± 8.7	70.2 ± 8.2	0.4014
Heart rate (HR)	71.1 ± 8.4	72.0 ± 7.1	0.5197
Waist to hip ratio	0.8 ± 0.11	0.76 ± 0.7	**0.0143**
Fasting blood glucose (mmol/l)	4.9 ± 0.54	4.8 ± 0.73	0.4647
Triglycerides (mmol/l)	0.83 ± 0.43	0.92 ± 0.36	0.1847
Total cholesterol (mmol/l)	4.38 ± 0.68	4.56 ± 0.82	0.1525
HDL cholesterol (mmol/l)	1.51 ± 0.36	1.52 ± 0.36	0.8098
Insulin Conc. (mu/l)	3.85 ± 2.15	4.5 ± 2.0	0.0616
HOMA-IR	0.84 ± 0.45	0.98 ± 0.43	0.1051
C-Peptide	1.27 ± 0.33	1.25 ± 0.33	0.7577
Total Calories/day (kcal)	2181 ± 588	1972 ± 540	**0.0449**
Carbs (g)	272.4 ±76.5	256.8 ±1 11.9	0.3750
Fat (g)	68.5 ± 31	55 ± 25.7	**0.0324**
Protein (g)	99.3 ± 42.8	81.9 ± 30.3	**0.0348**
Chol (mg)	226.6 ± 148.2	**155.3 ± 111.2**	**0.0139**
Sodium (mg)	1375.8 ± 397.7	**1450.4 ± 523.5**	0.4494
Sugars (g)	106.2 ± 91.5	65.7 ± 39.1	**0.0120**
Fiber (g)	25.3 ± 17.5	19 ± 14.9	0.0790

4.2 Physical evaluations

In the physical activity laboratory, a standard protocol was used to carry out all anthropometric assessments for all participants wearing tight fitting cloths and by using the same equipment throughout the study. Measurements were made to the nearest 0.1 unit. Height (cm) was taken by instructing the volunteer to stand feet together, back and heels against the upright bar of the height scale. Head was also positioned upright against the backboard. The volunteer was requested to take a deep breath as the investigator applied a gentle, upward pressure under the angle of the mandible. Other investigator slid down the horizontal bar attached to the scale snugly close on the examinee's head and measurements were taken. Body weight (kg) was measured using a beam balance and BMI was

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calculated as follows: BMI = weight (kg)/height (m2). Waist and hip circumferences (cm) were measured in duplicate using non-elastic tape. Waist circumference was measured at the minimum circumference horizontally between the iliac crest and the rib cage while the hip circumference was measured at the maximum protuberance of the buttocks, and the waist to hip ratio was calculated. The same investigator performed these measurements for all volunteers on every occasion. Whole body composition including percent body fat, soft lean mass, and total body water were measured using an IOI 353 Body Composition Analyzer (Jawon Medical, South Korea).

4.3 Physical activity measurements

All participants in this study were given an electronic accelerometer (Actigraph GT3X; Actigraph LLC, Pensacola, FL, USA) to measure daily physical activity (PA) levels. Subjects were advised to maintain their normal daily habitual PA levels during the study period. The accelerometers were attached to an elasticized belt and worn on the right hip for seven consecutive days (except when bathing and during water activity). The accelerometer provided PA measurements that included activity counts, vector magnitude, energy expenditure, step counts, PA intensity levels, and metabolic equivalents of tasks **(**METs). A one-minute epoch was used in this study, with activity counts assessed at 1-min intervals to ensure that the data quality for the participants included at least four days in which the accelerometer was worn for at least 60% time of the day. A non-wear time was taken as any block of time greater than or equal to 60 min wherein the activity count was equal to zero.

Freedson's cut-offs [40] were used to differentiate between the PA intensity levels, including light-intensity activity (100–1951 counts/min), moderate-intensity activity (1952-5724 counts/min), and high-intensity activity (> 5725 counts/min). All counts lower or equal to 99 counts/min were considered as sedentary. The data were also expressed as the mean intensity for each activity during the monitoring time (total accelerometer counts per total monitoring time).

4.4 Measurement of metabolic and inflammatory markers

Volunteers were asked for a second visit after an overnight fast of at least 10 hours. Three blood pressure measurements, along with the heart rate, were taken for each participant. Blood samples were collected in 10 mL EDTA tubes (BD Vacutainer system, Plymouth, UK). Plasma was separated and frozen immediately at -80 °C for further analysis. Total blood glucose, fasting plasma insulin, cholesterol, HDL-cholesterol, and triglycerides were determined by biochemical analysis using a single assay upon the completion of the sampling. Quality control sera were used to monitor the accuracy and precision of the assays.

Quantitative insulin sensitivity indices, HOMA-IR and HOMA-β, were calculated as follow.

HOMA-IR = fasting insulin (μU/L) × fasting glucose (mmol/L)/22.5

HOMA-beta cell function (HOMA-β) % = 360 x fasting insulin (μU/mL) / (fasting glucose (mg/dL) - 63)

4.5 Dietary Monitoring and Analysis

All participants were given food diaries and were instructed to weigh and record their daily intake of food and drinks on electronic scales (Salter Housewares, Kent, United Kingdom). A visual demonstration of how to use scales and diaries was given to each individual prior to the start of the study. All individuals were advised to maintain their

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normal dietary intake. Diaries were completed prior to the second visit. Food diaries' data were analyzed using CompEat pro (Nutrition systems, Banbury, United Kingdom) and an average of the daily nutrient intake was calculated.

4.6 Enzyme-Linked Immunosorbent Assay (ELISA)

Commercially available ELISA kits were used for detection of plasma levels of fasting insulin and C-peptide (Mercodia, Uppsala, Sweden), following instructions from the manufacturers.

4.7 Determination of plasma cytokines/chemokines

A total of 41 cytokines and chemokines were measured using panel MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel-Premixed 41 Plex-Immunology Multiplex Assay (Milliplex map kit, HCYTMAG-60 K-PX41; Millipore, USA), following the manufacturer's instructions. Data from the reactions were acquired using Luminex, Milliplex analyzer while a digital processor managed the data output and Milliplex analyst software was used to determine the mean fluorescence intensity (MFI) and analyte concentration (pg/mL).

4.8 Statistical Analysis

Data were analyzed using SPSS version 25 (SPSS, Inc., Chicago, IL) and Graph Pad Prism 7.01 (version 6.05; San Diego, CA, USA) and expressed as the mean ± standard deviation (SD). Group means were compared using either a two-tailed t-test when comparing between two groups or one-way ANOVA if more than two groups were analyzed. Linear relationships between two variables were assessed by determining Pearson's correlation coefficient "r" values. A chi-squared test and Pearson's correlation were used to assess the direction and strength of the association between energy intake from carbohydrate and immune-metabolic parameters. The equality of distributions or data normality testing was done by Shapiro–Wilk test which has the best power for a given significance. All P-values ≤0.05 were considered statistically significant.

5. Conclusions

The presented data highlight, for the first time, the effect of low carbohydrate intake on factors related to IRS in normal-weight population. We have shown that the individuals consuming less than 45% of their daily energy intake from carbohydrates had dysregulated glucose homeostasis, driven by elevated plasma C-peptide levels **(Figure 4)**.

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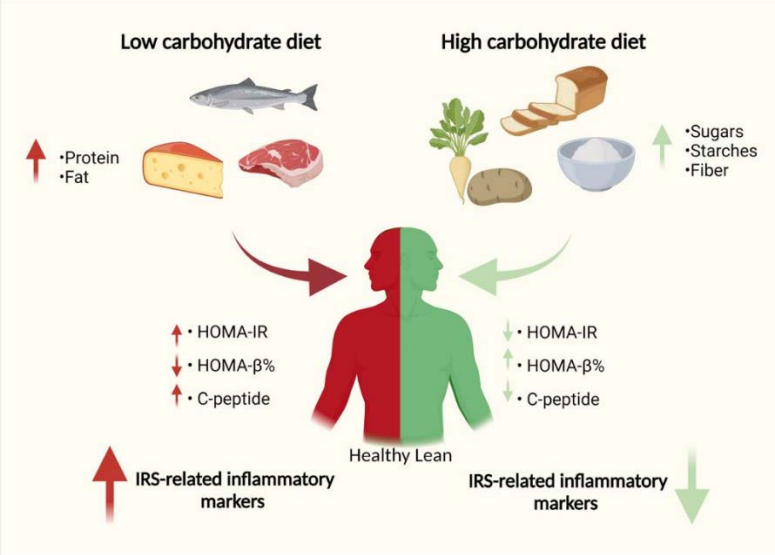

Figure 4 Schematic illustration. Low carbohydrate diet in healthy weight indivutules is assoistaed with disregulated glucose homeostasis, driven by elevated plasma C-peptide levels.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; The assessment of total energy from dietary intake divided by dietary source.

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Institutional Review Board Statement: The study was approved by the Kuwait Ministry of Health (MOH) Ethics Board (2017/542).

Informed Consent Statement: All participants were informed about the study and the risks involved, before signing a written consent form and doing a health screen.

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