Decreased insulin secretion and incretin concentrations and increased glucagon concentrations after a high-fat meal when compared with a high-fruit and -fiber meal

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OUR PREVIOUS WORK ON POSTPRANDIAL INFLAMMATION after a high-fat meal indicated that the magnitude of the increase in insulin concentrations after such a meal may be lower than that after an equicaloric American Heart Association (AHA) meal rich in fruit and fiber (10). Consistent with this, plasma glucose concentrations following the HFHC meal were also higher than those after the AHA meal. Furthermore, the consumption of fresh orange juice with a HFHC meal not only prevents oxidative and inflammatory stress but is also associated with an increase in the insulin concentration generated after such a meal (12). Since this potentially interesting observation was made accidentally following a protocol in which blood samples were obtained at hourly intervals, we decided to confirm this observation and explore it further by obtaining frequent blood samples.

CD26 is a proinflammatory membrane protein that is distributed ubiquitously in different cell types, including peripheral blood mononuclear cells (MNC) (19). It has a high turnover and is shed into plasma, where it has a peptidase activity and is called dipeptidyl peptidase-IV (DPP-IV). This enzyme has avidity for the two major incretins, glucagon-like peptide (GLP)-1 and gastric insulinotropic polypeptide (GIP), and rapidly hydrolyzes them such that their half-life in plasma is 2 and 5 min, respectively (13). Thus, plasma DPP-IV level determines the stability of incretins in plasma. Due to DDP-IV activity, only about 50% of the secreted incretins get to the pancreatic β-cells to induce insulin secretion (14).

On the basis of the above findings, the hypotheses tested were as follows: 1) a HFHC meal induces a smaller increase in insulin, C-peptide, and proinsulin concentration and a greater increase in glucose concentration than an isocaloric high-fat and -fiber AHA meal; 2) the smaller increase in insulinogenesis following a HFHC meal is associated with a smaller increment in plasma concentrations of the incretins GIP and GLP-1; and 3) there is a concomitant increase in CD26 expression in peripheral blood MNC and plasma DPP-IV and glucagon concentrations after a HFHC meal.

SUBJECTS AND METHODS

Subjects. Ten normal-weight subjects [6 males; BMI 22.8 ± 0.7 (20.2–24.1) kg/m2, age 33 ± 4 (24–48) yr] participated in this single center prospective, open-label, randomized crossover study. All subjects were healthy, nonsmokers, not on any supplements or medications, including nonsteroidal anti-inflammatory drugs with normal lipid and metabolic profiles, and not pregnant. The subjects were randomized (1:1 ratio by study coordinator using the Excel randomization function of Microsoft Office) to consume 910 kcal of either a HFHC meal [egg muffin and sausage muffin sandwiches and 2 hash browns, which contained 88 g of carbohydrates, 51 g of fat (33% saturated), 34 g of protein, and 12 g of dietary fiber (calories from carbohydrates 41%, protein 17%, and fat 42%)] or an AHA-recommended meal [consisting of oatmeal, milk, orange juice, raisins, peanut butter, and an English muffin containing 21 g of dietary fiber (calories from carbohydrates 58%, protein 15%, and fat 27%)] on the first visit followed by the other meal on the second visit 1 wk later. Meals were consumed over 20 min. Blood samples were collected...
before and at 15, 30, 45, 60, 75, 90, 120, 180, and 300 min following completion of the meal in tubes containing protease inhibitor cocktail (Sigma, St. Louis, MO) and DPP-IV inhibitor (EMD Millipore, Billerica, MA) for incretins and pancreatic peptide measurement. Additional blood samples were collected prior to and at 1, 2, 3, and 5 h following the intake of the food and used for cellular work. The experimental protocols were approved by the Human Research Committee of the State University of New York at Buffalo, and each subject signed an informed consent form.

**MNC isolation.** Blood samples were collected in Na-EDTA as an anticoagulant. Three to five milliliters of anticoagulated blood sample were carefully layered over 3.5 ml of Lympholyte medium (Cedarlane Laboratories, Hornby, ON, Canada) and centrifuged to separate the cells. The top band consisted of MNC and was carefully collected. This method provided yields of >95% pure MNC suspensions.

**Western blotting.** MNC total cell lysates were prepared, and electrophoresis and immunoblotting were carried out as described before (2). Polyclonal or monoclonal antibodies against CD26 (Abcam, Cambridge, MA) and actin (Santa Cruz Biotechnology, Santa Cruz, CA) were used, and the membranes were developed using West Femto chemiluminescence reagent (Pierce Chemical, Rockford, IL). Densitometry was performed using molecular analyst software (Bio-Rad, Hercules, CA), and all values were corrected for loading with actin.

**Reactive oxygen species generation measurement by chemiluminescence.** Five-hundred microliters of MNC (2 × 10^6 cells) were delivered into a Chronolog Lumi-aggregometer cuvette. Luminol (Hercules, CA), and all values were corrected for loading with actin.

**Total RNA isolation and real-time RT-PCR.** Total RNA isolation and RT-PCR were performed as described previously (4, 11). Expression of CD26, TNFα, and IL-1β mRNA was measured. The specificity and the size of the PCR products were tested by adding a melt curve at the end of the amplifications and by running it on a 2% agarose gel. All values were normalized to the expression of three housekeeping genes (β-actin, ubiquitin C, and cyclophilin A).

**Plasma measurements.** Three milliliters of blood was collected in tubes containing DPP-IV inhibitor and protease inhibitor cocktail before and at 15, 30, 45, 60, 75, 90, 120, 180, and 300 min following meal consumption. Glucose levels were measured in plasma by ySI 2300 STAT Plus glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Enzyme-linked immunosorbent assays were used to measure insulin, C-peptide, proinsulin, active GLP-1, total GLP-1, GIP, and peptide YY (Sigma, St. Louis, MO) as well as DPP-IV and glucagon (R & D Systems, Minneapolis, MN). Plasma lipopolysaccharide (LPS) concentration was measured by a commercially available kit (Cambrex Limulus Amebocyte Lysate kit; Lonza, Walkersville, MD). Plasma samples used for LPS determination were stored in LPS-free glass tubes to prevent loss of LPS to the plastic tube wall. All materials used for the assay were rendered LPS free. Plasma was diluted 10-fold and heated to 75°C for 5 min prior to LPS measurement. C-reactive protein was measured using ELISA assay (American Diagnostica). Free fatty acid (FFA) levels were measured by a colorimetric assay (Wako Chemicals, Richmond, VA). Thiobarbituric acid-reactive substance (TBARS) was assessed in plasma using a fluorescence assay (Zeptometrix, Buffalo, NY).

**Statistical analysis.** Statistical analysis was conducted using SigmaStat software version 3.1 (SPSS, Chicago, IL). Data are represented as means ± SE. Percent change from baseline was calculated, and statistical analysis for change from baseline was carried out using one-way repeated-measures analysis of variance (RMANOVA) followed by Holm-Sidak post hoc test. Two-factor RMANOVA analysis followed by Tukey’s post hoc test was used for all multiple comparisons between different groups. Area under the curve (AUC) for glucose, insulin, and incretins was calculated for each subject’s samples and compared between meals by paired t-test. In view of the marked variation in the data related to glucose concentrations, log transformation was carried out prior to statistical comparisons. Spearman rank order correlation was used to test the relation between changes in DPP-IV concentrations following both meals and changes in other end points tested. By conservatively assuming a mean difference in DPP-IV concentrations of 20% between the two groups and a standard deviation of 15%, a sample size of 10 was sufficient to obtain a statistically significant difference (P < 0.05) between the two groups with a paired test power of 0.8.

**RESULTS**

**Plasma glucose and insulin concentrations.** The intake of HFHC and AHA meals induced significant peak increases in glucose concentration from 83 ± 3 to 106 ± 7 mg/dl at 30 min (P < 0.05) and from 81 ± 3 to 107 ± 3 mg/dl at 15 min (P < 0.05), respectively. Following the initial peak, glucose levels following the AHA meal were generally lower throughout the followup period and were significantly lower at 1 h (82 ± 5 vs. 94 ± 6 mg/dl, P < 0.05) compared with the HFHC meal (Fig. 1A). The AUC of glucose concentrations following the AHA meal was significantly lower than that after the HFHC meal after log transformation (Fig. 1B). This was associated with greater insulin secretion following the AHA meal throughout the monitoring period. Insulin concentrations were significantly higher (P < 0.05 by paired t-test) at 1 h following the AHA meal (from 4.4 ± 0.8 to 25.2 ± 2.5 μU/ml) compared with the HFHC meal (from 3.8 ± 0.5 to 19.7 ± 1.7 μU/ml) and when AUCs were compared (P < 0.05, paired t-test) (Fig. 1, C and D). The ratio of insulin to glucagon (μU/mg) was significantly greater after the AHA meal compared with that after the HFHC meal (P < 0.05, 2-way RMANOVA and paired t-test for AUC; Fig. 1, E and F). C-peptide concentrations increased significantly following both meals (from 0.78 ± 0.3 to a peak of 2.5 ± 0.5 ng/ml at 60 min following the HFHC meal and from 0.71 ± 0.3 to 3.5 ± 0.4 ng/ml at 45 min following the AHA meal). Peak increase and the AUC of the increase were significantly higher by 54 ± 19 and 34 ± 12%, respectively, following AHA meal (P < 0.05; Fig. 1, G and H). Proinsulin also increased significantly following both meals (from 3.2 ± 0.7 to a peak of 20.9 ± 3.8 pM at 90 min following the HFHC meal and from 3.7 ± 0.9 to 23.7 ± 4.1 pM at 90 min following the AHA meal). AUC of the increase was significantly higher by 22 ± 8% following the AHA meal (P < 0.05; Fig. 1, I and J). There was a slightly higher insulin/C-peptide ratio peak increase following the HFHC compared with the AHA meal (from 0.10 to 0.23 vs. 0.14 to 0.22 pmol/pmol; not significant), but the ratio was similar when AUCs for both insulin and C-peptides were used (0.126 vs. 0.121; not significant). There was no significant difference in proinsulin/insulin ratio between the two meals (data not shown).

Plasma FFA concentrations fell significantly after both meals (from 0.37 ± 0.05 to a nadir of 0.21 ± 0.02 mM at 60 min following the HFHC meal and from 0.37 ± 0.03 to a nadir of 0.11 ± 0.01 mM at 60 min following the AHA meal). The magnitude of the fall was significantly lower (49 ± 7 vs. 70 ± 3%, P < 0.01) after the HFHC meal than after the AHA meal (Fig. 1K).

**Plasma incretins and glucagon concentrations.** HFHC meal intake induced a significant increase in postprandial glucagon
concentrations that peaked at 2 h (from 101 ± 14 to 183 ± 30 pg/ml, P < 0.05) compared with the AHA meal (from 113 ± 9 to 143 ± 18 pg/ml, P < 0.05; Fig. 2A). The change in glucagon concentrations was significantly different between the two meals (AUC P < 0.05; Fig. 2B).

The AUC of the postprandial concentrations of total and active GLP-1 was not significantly different between the two meals. However, there was an early (at 15 min) and brief peak of GLP-1 concentrations following the AHA meal, but there was no difference in the AUC following the two meals (Fig. 2, C–F). This early peak coincides with the early peak in insulin secretion. The GIP response was significantly lower following HFHC meal intake (from 28 ± 2 to 212 ± 35 pg/ml, P < 0.05) compared with the AHA meal (from 23 ± 2 to 253 ± 38 pg/ml, P < 0.05; Fig. 2G) at 75 min, which continued throughout the experimental period of 300 min. GIP AUC was significantly lower following the HFHC meal compared with AHA meal (P < 0.05 by paired t-test; Fig. 2H). The HFHC meal induced a significantly higher peak PYY response at 45 min compared with the AHA meal (from 120 ± 13 to 189 ± 16 pg/ml vs. from 127 ± 15 to 153 ± 13 pg/ml, respectively, P < 0.05; Fig. 2I). However, there was no significant difference between the two meals when AUCs of PYY were compared (Fig. 2J).

Fig. 1. Change in concentrations and area under the curve for glucose (A and B), insulin (C and D), insulin/glucose ratio (E and F), C-peptide (G and H), proinsulin (I and J), and free fatty acids (FFA; K) following 910-kcal high-fat/high-carbohydrate (HFHC) and American Heart Association (AHA) meals in normal subjects (n = 10 for all). Data are presented as means ± SE. #P < 0.05 with paired t-test, comparing HFHC with AHA.
Oxidative stress and inflammatory mediators. Oxidative stress measured as ROS generation by MNC and as TBARS concentration in plasma increased significantly (by 84 ± 19 and 34 ± 10%, respectively, at 3 h, P < 0.05) following HFHC meal, whereas it did not change following AHA meal (Table 1). Plasma LPS concentrations increased significantly by (by 58 ± 10%, P < 0.05) over the baseline (from 0.38 ± 0.02 to 0.59 ± 0.03 EU/ml at 5 h, P < 0.05) following the HFHC meal. The intake of AHA did not induce any significant change in LPS levels. The intake of the HFHC meal also induced significantly higher expression of TNFα (by 36 ± 10%) and IL-1β (by 49 ± 12%) mRNA at 3 h compared with the AHA meal (P < 0.05; Table 1). There was no significant change in C-reactive protein concentration following either meal.

Plasma DPP-IV concentrations. The intake of the HFHC meal induced a significant increase in plasma concentration of DPP-IV from 341 ± 28 to 416 ± 31 ng/ml at 2 h, an increase of 18 ± 4%, whereas it did not change following the AHA meal (366 ± 34 to 375 ± 35 ng/ml; Fig. 3A). There was a significant difference in DPP-IV concentrations following the two meals (P < 0.05, 2-way RMANOVA).

CD26 expression in MNC. There was a significant increase in CD26 mRNA expression in MNC by 37 ± 9% at 2 h following the HFHC meal (P < 0.05, RMANOVA), whereas there was no significant change in CD26 mRNA expression following the AHA meal (Fig. 3B). CD26 protein levels in MNC increased significantly by 23 ± 8% above the baseline at 3 h (P < 0.05) when the HFHC meal was ingested, whereas there was no significant alteration in CD26 protein after the AHA meal (Fig. 3, C and D). There was a significant difference in CD26 mRNA and protein expression following the two meals (P < 0.05, 2-way RMANOVA).

Relation between DPP-IV, LPS, and glucagon. The change in DPP-IV concentration at 2 h following the HFHC and AHA meals was significantly related to the changes in plasma LPS concentrations at 5 h (r = 0.436, P = 0.012) and glucagon concentrations at 2 h (r = 0.374, P = 0.034) (Fig. 4).

DISCUSSION

Our data clearly show that the magnitude of increase in insulin, C-peptide, and proinsulin following the HFHC meal was significantly lower than that after the AHA meal. Consistent with this, the increase in plasma glucose concentrations was significantly greater (AUC) after the HFHC meal. As a result, insulin/glucose ratio was also lower after the HFHC meal.
meal. Thus, insulinogenesis after the HFHC meal was diminished despite higher glucose concentrations after the HFHC meal. Clearly, there are additional factors other than glucose concentrations that are also responsible for insulinogenesis.

The data on GLP-1 concentrations for both total and active forms show that the early peak at 15 min after the AHA meal was prominent, whereas there was no peak after the HFHC meal. The AUC of GLP-1 following the two meals was not significantly different. The early peak may be important in inducing the early peak of insulin after the AHA meal, whereas its absence after the HCFC meal may account for the absence of the early peak of insulin. The AUC for GIP was smaller after the HFHC meal than after the AHA meal; the difference in insulin secretion, which starts early, cannot be accounted for by the relatively late increases in GIP after the AHA meal. However, they may account for the tendency for greater insulin concentrations late after the AHA meal.

The increase in plasma DPP-IV after the HFHC meal occurred at 2 h, and thus, it cannot account for the lack of the early peak of GLP-1. Although there was an increase in CD26 protein expression following the HFHC meal and not the AHA meal, this increase occurred at 3 h after the meal, whereas the increase in DPP-IV occurred earlier at 2 h, and thus it could not contribute to it. The increase in the expression of CD26 mRNA occurred at 2 h, 1 h before the increase in CD26 protein. It is possible that the more prolonged increase in glucose concentrations following the HFHC meal and its greater fat content may have contributed to the higher DPP-IV levels and CD26 expression following the HFHC meal since glucose is known to be proinflammatory and to induce oxidative stress (1, 3). There are recent data showing that plasma DPP-IV is increased in the obese (18).

There was a clear increase in plasma glucagon concentrations after the HFHC meal. There was a trend toward an increase in glucagon after the AHA meal, but this was not significant with a rapid reversal toward the baseline. AUC for glucagon was significantly greater after the HFHC meal. This would contribute to the higher glucose concentrations after the HFHC meal since glucagon induces glycogenolysis and hepatic glucose production (5, 15). It is of interest that the increase in glucagon concentrations was related significantly to the increase in DPP-IV levels. It is not known whether glucagon induces inflammation or inflammation induces glucagon. On one hand, it is known that fat intake induces glucagon secretion (20).

Indices of oxidative and inflammatory stress showed an increase after the HFHC meal as expected. ROS generation, TBARS concentration, and the expression of TNFα and IL-1β mRNA increased significantly at 1 h for all, except for ROS generation, which increased significantly at 2 h. This pattern is consistent with that of CD26 mRNA expression, which increased at 2 h. Thus, the increase in CD26 expression is probably a part of the comprehensive proinflammatory response following the HFHC meal. In contrast, the increases in the expression of TNFα and IL-1β that occurred later were much smaller in magnitude and were transient. It is relevant in the context of β-cell function and insulinogenesis that IL-1β is known to be toxic to β-cells (8) and that the administration of the IL-1 receptor antagonist restores β-cell function and improves insulin secretion and hyperglycemia in type 2 diabetes (17).

Equally important is that whereas the HFHC meal induced an increase in LPS concentration, the AHA meal did not. The increase in LPS concentration was significantly related to the increase in DPP-IV levels in plasma. LPS administration has been shown previously to induce insulin resistance (6), and since IL-1β and TNFα, stimulated by LPS, induce suppressor cytokine signaling-3 (SOCS-3), which interferes with insulin signal transduction (9, 16, 21), these actions may promote insulin resistance. HFHC meal intake also induces SOCS-3, Toll-like receptor (TLR)-4, and TLR-2, which also mediate insulin resistance (10, 22). Thus, a proinflammatory HFHC meal not only reduces insulinogenesis but also lays the foundation for insulin resistance.

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**Table 1. Change in oxidative stress and inflammatory mediators following 910-kcal AHA or HFHC meal in normal subjects**

<table>
<thead>
<tr>
<th>Meal</th>
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<th>2</th>
<th>3</th>
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<tr>
<td>MNC ROS generation, mV</td>
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<tr>
<td>AHA</td>
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<td>135 ± 25</td>
<td>142 ± 28</td>
<td>144 ± 25</td>
<td>132 ± 21</td>
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<td>HFHC</td>
<td>134 ± 22</td>
<td>172 ± 29</td>
<td>221 ± 57*</td>
<td>247 ± 39*</td>
<td>198 ± 33*</td>
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<td>TBARS, nM</td>
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<tr>
<td>AHA</td>
<td>1.25 ± 0.20</td>
<td>1.29 ± 0.31</td>
<td>1.21 ± 0.19</td>
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<td>1.20 ± 0.18</td>
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<tr>
<td>HFHC</td>
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<td>1.48 ± 0.21*</td>
<td>1.55 ± 0.25*</td>
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<tr>
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<td>AHA</td>
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<td>HFHC</td>
<td>0.54 ± 0.12</td>
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<tr>
<td>AHA</td>
<td>0.58 ± 0.08</td>
<td>0.68 ± 0.16</td>
<td>0.73 ± 0.23</td>
<td>0.84 ± 0.22*</td>
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<td>HFHC</td>
<td>0.53 ± 0.07</td>
<td>0.85 ± 0.17*</td>
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<td>1.19 ± 0.4</td>
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<td>AHA</td>
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<td>0.46 ± 0.03</td>
<td>0.53 ± 0.04*</td>
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Data are represented as means ± SE; n = 10. AHA, American Heart Association; HFHC, high-fat/high-carbohydrate; MNC, mononuclear cells; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substance; CRP, C-reactive protein; AU, arbitrary units. *P < 0.05 compared with baseline with 1-way repeated-measures ANOVA; #P < 0.05 between groups with 2-way repeated-measures ANOVA.
In conclusion, HFHC meal intake leads to a significantly smaller increase in insulin, C-peptide, and proinsulin concentrations and a more prolonged increase in glucose concentrations than those after an AHA meal. In addition, the early peak in GLP-1 concentration is absent, and there is an increase in plasma DPP-IV levels and CD26 expression in MNC, endotoxemia, and inflammatory mediators following the HFHC meal compared with the AHA meal. Plasma GIP concentrations are also lower after the HFHC meal. HFHC meals may thus contribute to relatively diminished insulinogenesis and insulin resistance in the long term and contribute to the pathogenesis of diabetes.

Fig. 3. Change in plasma dipeptidyl peptidase-IV (DPP-IV) concentrations (A), CD26 mRNA expression (B), and representative Western blots of average changes in CD26 protein (C and D) in mononuclear cells (MNC) following 910-kcal HFHC or AHA meals in normal subjects (n = 10 for all). Data are presented as means ± SE. *P < 0.05 with repeated-measures ANOVA compared with baseline; #P < 0.05 with 2-way RMANOVA, comparing HFHC with AHA.

Fig. 4. Spearman Rank order correlation between changes in DPP-IV plasma concentration and changes in plasma LPS (A) and glucagon concentrations (B) following 910-kcal HFHC and AHA meals in 10 normal subjects.
REFERENCES


