Attention to metabolic hunger and its effects on *Helicobacter pylori* infection

Mario Ciampolini\textsuperscript{a,}\textsuperscript{*}, Lorenzo Borselli\textsuperscript{a}, Valerio Giannellini\textsuperscript{b}

\textsuperscript{a}Department of Pediatrics, University of Florence, 50132 Florence, Italy, \textsuperscript{b}Department of science farmaceutiche 50 121 Florence, Italy

Received 27 October 1999; received in revised form 10 February 2000; accepted 13 March 2000

Abstract

A significant decrease in the bacterial count of small intestinal mucosa has been observed in children with recurrent diarrhea or abdominal pain in the time that has elapsed from the previous meal. Humans may be trained to recognize metabolic feelings of hunger that are associated with a steady and slightly lower glycemia than baseline, between 4.7 and 3.9 mmol/L (intervention). An eating habit associated with a decrease in preprandial glycemia prevented diarrhea relapses, and was expected to impair intestinal microflora growth, including *Helicobacter pylori* in the stomach. The development of *Helicobacter pylori* infection might be prevented during childhood, and recovery from infection may be expected with intervention. The improvement in attention to metabolic feelings consisted of acquiring a predictive ability of glycemia by distinction between unsolicited hunger feelings (metabolic hunger) and those associated with external cues. Matching intake to the inbetween energy needs served to predict the subsequent emergence of the metabolic hunger. The matching was further compensated for the early or late emergence of metabolic hunger at the subsequent meals. Fruit and vegetables were increased to avoid abrupt glycemia lowering. This intervention was trained in 5-month periods. Subjects (209, 44, and 58) completed their training during 4-year periods between 1982 and 1994, and were enrolled in a prospective, controlled, randomized, interventional, preventive, and cohort study. The “prevention” hypothesis was tested in a subgroup of 86 healthy infants who were recalled in the years 1996 to 1998. An 8-year follow-up in the “prevention” study (\(p < 0.05\) and \(< 0.001\), respectively). A 4.7% seroprevalence of *H. pylori* infection was observed in the intervention group, with 30.2% in the control group at a mean age of 10 years after approximately an 8-year follow-up in the “prevention” study (\(p < 0.0005\)). The seroprevalence decreased to 9 of 24 (37.5%) under intervention as opposed to 20 of 23 controls (87%) in the recovery study (\(p < 0.002\)). A significant positive correlation was found between DAP glycemia and the anti-*H. pylori* serum antibody concentration (\(r = 0.52; p = 0.0002\)). A decrease in the level of immune stimulation by *H. pylori* infection was observed due to the intervention, which may have a preventive and therapeutic role on the infection. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: *Helicobacter pylori*; Insulin resistance; Fasting glycemia; Ingestive behavior; Chronic diarrhea; Energy intake; Vegetable intake

1. Introduction

Insulin sensitivity measures the individual current carbohydrate (and indirectly energy) storage rate from blood to the body cells. The measured value has a range as wide as three times the lower range limit in people with normal glucose tolerance [1–7], and even more in a mixed sample under our observation [8]. Vascular and ovary risks and progressive deterioration develop in association with the slowest rates (insulin resistance), despite absence of symptoms for many years [2, 3, 5–7, 9, 10]. A (cognitive) feeding based on recognition of both energy intake and current insulin sensitivity may improve insulin sensitivity and prevent all of these widespread risks. A feeding on demand (hunger) was proposed in infants to minimize immune stimulation by bacterial growth in intestine [8, 11–13], and was revealed to be associated with insulin sensitivity increase [14]. The hunger expression by the infant was also associated with a decrease in preprandial glycemia, and followed by a decrease in resting metabolic rate, which suggests a metabolic

\* Corresponding author. Tel.: 011 39 55 575037; Fax: 011 39 55 570380

E-mail address: ciampolini@cesif.unifi.it

0031-9384/00/$ – see front matter © 2000 Elsevier Science Inc. All rights reserved.

PII: S0031-9384(00)00273-0
origin [8,11–13,15,16]. The infant expresses the same manifestation of hunger as soon as he/she sits down at the table, at the sight of food, or even by the speech of older children. These external stimuli usually are more powerful than the metabolic hunger [17,18]. This distinction was learned by attentive caregivers whose infants were addressed to our unit to acquire subjective improvements, for example, prevention of mild intestinal complaints or increase in fitness [8,11–16,19]. The use of metabolic hunger as a guide in feeding is currently explored in infants in a controlled, randomized, follow-up, which has been maintained for about 18 years. This prospective study might show unknown effects of the intervention, for example, on intestinal microflora, including *Helicobacter pylori* (*H. pylori*) in the stomach. The development of this infection might be prevented during childhood, and recovery may be expected under intervention.

The “prevention” hypothesis was tested in a subgroup of the cohorts under recall in the years 1996 to 1998. Eighty-six children were negative for *H. pylori* antibody in the second year of life, and their frozen sera were available in the follow-up at various periods of time from enrollment. A final blood sample was obtained and analyzed for *H. pylori* infection.

The study was extended into the adult age range [8,20]. The metabolic hunger was ignored in older children and adults, and a training period was necessary for learning recognition. The metabolic hunger may be felt in the previous hour and remembered upon going to the meal table, whereas the “external” hunger appears only at events associated with food. The “recovery” hypothesis was investigated in newly enrolled subjects between 5 and 25 years of age, who were positive for anti- *H. pylori* and had no need for an immediate antibiotic treatment at baseline. A single blood test assessed the outcome after 6 to 18 months: either negative seroconversion or maintenance of seropositivity. Preprandial glycemia measurement was used for apprehension of recognition of metabolic hunger, and for assessment of the compliance under intervention (causal factor). Home diaries reported foods and Yes or No hunger manifestation or perception with preprandial glycemia measurements, and documented subjects’ voluntary compliance with the intervention. Anthropometric measurements verified positive growth. The pre–post decrease in glycemia may both prove actual recognition of metabolic hunger and be associated with recovery from the *H. pylori* infection as assessed by seroconversion.

2. Materials and methods

2.1. Study design

The intervention and the controlled follow-up were designed in 1980, and the intervention was reproducible by complying with a main variable and a support variable [8,11–16,19]. The main variable was dependent, and was assessed by both subjective evaluation and biochemical measurement. The assessment consisted of the recognition of the blood glucose level by body feelings of bearable hunger and preprandial glycemia measurement that was maintained between 3.9 and 4.7 mmol/L. The subjective evaluation served permanently after a period of training and glycemia measurements for periodical verification. The intervention predicted or manipulated a few independent variables to influence the dependent main variable. Snacks, climate, and energy density in the meal were independent. The decrease in energy intake reproduced a variable part of compliance, which might be substituted by an increase in physical exercise. All independent variables could be substituted by a variable acting in the same direction to comply. Two independent variables with opposite effects on glycemia might be decreased or increased together to obtain the same blood glucose concentration. Therefore, all independent variables expressed only a variable part or negligible amount of compliance. The support variable is more understandable, and erroneously considered the only compliance. Abundant vegetable intake is necessary to prevent hypoglycemia events in hyperinsulinemic patients waiting for metabolic hunger. On the other hand, a high vegetable intake when glycemia is high is associated with the development of diarrhea, abdominal pain, or headache [8,12,15,19]. Compliance with only one of the two prescriptions was associated with these undesirable effects, whereas the two prescriptions together produced bearable hunger for about 3 h in adults, i.e., the possibility of taking the subsequent meal before glycemia drops [8,12,15,21–24].

Phone calls were repeated at least for 7 initial days. Detailed descriptions of metabolic hunger, food intake, and physical and social activity patterns were collected throughout the 5-month observation period. Seven-day home diaries, anthropometric measurements, and clinical evaluations were obtained at baseline, every 50 days for the 5-month initial apprehension, and every year during subsequent follow-up. Diaries reported hunger recognition, food intake, and also preprandial blood glucose measurements at baseline and after 8 year of age.

The intervention required a certain amount of motivation, attention, and discipline, and therefore, it recruited persons who were willing and able to fulfill the protocol after 1986. Willing caregiver–children pairs in which the children were in their second year of life were randomly assigned to two open cohorts for a controlled, prospective investigation, and were recalled in alphabetical (i.e., random) order every 4 years (Table 1). An equal number of consecutive subjects were matched for age and length of follow-up, and antibody to *H. pylori* were added to programmed tests between the years 1996 and 1998 [8,11,12]. The seroconversion to *H. pylori* was the outcome. These were measured in 86 subjects with different lengths of follow-up. Newly recruited subjects between 5 and 25 years were selected for the “recovery” study. The same recruitment and 1:1 random assignment were employed, and the same alphabetical (random) recall was used for end-trial assessment after 6 to 18 months under intervention.

2.2. Subjects

In early 1987, the Pediatric Gastroenterology Unit of Florence University limited recruitment to patients who
were addressed and prepared for the intervention by interpersonal relationships or their physician. The Hospital Gastroenterology Unit served the outpatients. Healthy subjects, 6 to 60 months of age were eligible. The clinical assessment of all patients consisted of a routine physical examination and of diagnostic biochemical and microbiological evaluations to rule out disorders such as celiac disease, lactose intolerance, cystic fibrosis, inflammatory bowel disease, liver disease, pancreatitis, and bacterial and parasitic infections [15,16,19]. Those who had organic diseases, acute or relapsing conditions, or were unmotivated or unreliable were excluded (see Validations). The subjects were healthy as judged from reactive C protein, white blood count, and absence of important intestinal or other organ complaints. The infants were concurrently assigned to the intervention or control group, i.e., the “external induction” or “ad lib” feeding group, by use of a concealed sequence prepared on a random-number table at diagnosis between 1982 and 1994 [25]. The concealed sequence was prepared every 2 years, with an initial ratio of 2:1 between intervention and control subjects. This ratio was changed to 1:1 at the beginning of 1987, and again to 2:1 in 1991, for noncontrolled research on resting metabolic rate. Table 1 reports the number of examinations every 4 years in comparison to the number of subjects who completed the first 5-month period, which was educational in the intervention group. The difference shows the 4-year dropouts. Obtaining a diagnosis, medical verification, and psychological support motivated the caregivers of the control group. Dropouts in both groups were motivated by the absence of motivating illness, lack of time or money, and well-being. Sufficient apprehension or distrust to novelty caused renounce to follow-up in the intervention group. Eighty-nine children were alphabetically recalled for the last compliance and blood tests in both groups. Of these, 86 were examined.

The intervention and control group for the “recovery” investigation were formed in subjects aged from 60 months to 25 years by the same random method in a 1:1 proportion between 1991 and 1996. No antibiotic treatment was used for H. pylori elimination because of the absence of important dyspeptic symptoms. Four of 28 intervention subjects and 3 of 26 controls did not comply with instructions or follow-up in this study. The compliance was repeatedly assessed, and blood sampling was tested once in the 47 subjects 6 to 18 months after baseline. The hospital management works for profit under a newly implemented state law, and collection of data has stopped due to lack of immediate profit in 1998.

No child had had febrile disease, had used any drug or medication in the preceding 3 weeks, or was treated for H. pylori infection during the follow-up. The study was reviewed and approved by the departmental Human Trial Committee. Informed consent was obtained from the infants’ parents.

### Table 1

<table>
<thead>
<tr>
<th>Follow-up (years)</th>
<th>4–8&lt;sup&gt;a&lt;/sup&gt;</th>
<th>8–12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&gt;12&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under intervention</td>
<td>141/177</td>
<td>78/136</td>
<td>47/110</td>
</tr>
<tr>
<td>Controls</td>
<td>103/134</td>
<td>47/117</td>
<td>41/99</td>
</tr>
<tr>
<td>Dropouts in first 5 months</td>
<td>6/47 (13%)</td>
<td>5/31 (16%)</td>
<td>89/199 (45%)</td>
</tr>
<tr>
<td>Controls</td>
<td>2/19 (10%)</td>
<td>2/20 (10%)</td>
<td>51/150 (34%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Forty-one children under intervention and 17 controls completed the 5-month follow-up between 1990 and 1994, and are added to the 1982–1990 input.

<sup>b</sup> Twenty-six children under intervention and 18 controls completed the 5-month follow-up between 1986 and 1990, and are added to the 1982–1986 entries.

<sup>c</sup> One hundred ten children under intervention and 99 controls completed the 5-month initial follow-up between 1982 and 1986.

### 2.3. Intervention (recognition of “metabolic hunger”)

The subjects or the caregivers, i.e., the mother and father of the child under 13 years of age, received verbal, written, and phone tutorial daily instructions for 1–2 weeks, and developed two mental habits at mealtimes: (1) evaluation of metabolic feelings (manifestations); (2) planning the next emergence of metabolic hunger to maintain previous habits and allow for change. The planning was carried out by matching energy-dense food in the meal to the needs for the planned time interval [11–16].

The subjects ignored the first mealtime at first training attempt. They waited for the emergence of the unexplored personal feeling(s) of metabolic hunger or for manifestations in children, and measured glycemia at first perception. Metabolic hunger and glycemia lower than 4.7 mmol/L allowed 100 to 200 g of low energy-dense food and about half of previous high energy-dense food intake for the first meals. The amount of low energy-dense food was increased in 2–3 days to arrive at the full prescription (see later). High energy-dense food was increased in 1–2 days only after the third day to avoid intestinal complaints for high fiber intake. The metabolic hunger arose in unplanned moments in the first days of trials and errors. After the early days it became easier to let the metabolic hunger arise within the hour that the meal was cooked. There were concerns about how a situation could be created without the sight or smell of food, and the reflection on the behavior or feelings in the previous hour often was useful. The repetition of the same manifestation with the same blood glucose levels indicated the metabolic hunger. The emergence of these feelings or expressions was perceived at subsequent meals, and this became a habit. Ignoring the first signs of metabolic hunger, as well as loss of meals, was discouraged after the first early training days.

The mother (or father) often knew the all-day behavior of the infant, and easily developed the recognition of metabolic hunger. Crying in the first months of life, mood changes like loss of enthusiasm for playing, gestural or verbal requests, or searching for food without any “external” stimulus all were considered to be signs of metabolic hun-
ger in infants [26]. The metabolic perceptions of older subjects consisted of gastric pangs, feelings of emptiness, or mental activity impairment (no enthusiasm, difficulty in sustained mental concentration, irritability). The caregivers described the type, intensity, and persistence of hunger, as well as the delay in food consumption. They reported the preservation of food type choices, social obligations, good mood, physical, and mental effectiveness, and the usual (or increased) day-to-day physical activity.

The social obligations like parties and school catering had to be included in planning the intake amount and timing of previous and subsequent meals. The emergence of hunger at the midday communal eating was planned by matching energy intake to the needs for the interval at breakfast, and compensated by verification of the emergence at the subsequent dinner. After learning the recognition, no metabolic hunger before the meal was considered a factor, which prolonged the time before the next hunger emergence and suggested a decrease in energy-dense food. Avoiding snacks was suggested, but early hunger emergence had to be satisfied with fruit and adequate energy-dense food if needed. The prescribed intervention included the increase of energy expenditure because of the positive correlation between energy expenditure and lean body mass. Overheating and overclothing were decreased, while outdoors and gym activity increased [27]. The meal was planned again at subsequent mealtimes and consumed in large, small, or null amounts on the basis of metabolic hunger and planned activity for the next interval. Ignoring metabolic hunger, as well as loss of meals, was discouraged.

A few people expressed doubts about the intensity of the perception to be used as a signal for eating. Five adults were given two more specific verbal definitions on metabolic hunger in the following succession: work until emergence of feelings that disturb activity, and subsequently, work until initial emergence of feelings associated with glycemia under 4.7 mmol/L. The first definition was associated with four of five subjects with meals consumed when glycemia was as low as 2.5–3.3 mmol/L, physical and mental weakness for hours before feeding, and occasional fainting and mental activity impairment (no enthusiasm, difficulty in sustaining mental concentration, irritability). The second was associated with glycemia between 4.2 and 4.7 mmol/L, not impairment of intermeal activity, mood changes, food abstinence, or excessive intake. This sensation was suggested as the appropriate meal signal. Four types of metabolic feelings were identified: (1) satiety or no thought of food; (2) appetite or consumption of available food at perception of it; (3) bearable hunger feelings between 3.9 and 4.7 mmol/L; and (4) unbearable or bulimic hunger under 3.6–3.3 mmol/L.

High energy-dense food was considered to be food with over 60 kcal/100 g, and fruit and vegetables were low energy-dense foods. Fruit was defined as food under 45 kcal/100 g (apples, pears, and oranges) and vegetables under 30 kcal/100 g. Tables with the energy content of food items were provided [8,11–16,19]. Three hundred grams of leafy vegetables per meal were prescribed for children older than 8 years of age and adults. Fruit and vegetable intake was united under the name “Low energy-dense food” (LEDF). Vegetable dishes could be fresh or cooked and savored with tomato, onion, pepper, oil, and mixed with other food. The mother evaluated the savor as pleasant. Fruits and vegetables were usually given to children as the first dish, except in presence of intense hunger. Tables with the energy content of food items were given out.

2.4. Clinical assessments and measurements

2.4.1. Clinical assessments

Clinical assessments were performed for diagnostic purposes at baseline, one to three times within 5 months of initial instructions, and every following year. The purpose was to evaluate each subject’s clinical condition and assess growth in children, changes in fat, and voluntary compliance with the study protocol.

Clinical assessments at baseline and at the end of protocol included standard hematological evaluations and urinalysis, urine culture, and examination of stools for occult blood, ova and parasites, antibodies to H. pylori, and bacterial cultures for potential pathogens. Children were examined to rule out other organic disorders, as previously noted. Comprehensive biochemical profiles were obtained on all children. Measurements included reactive C protein, serum albumin, hemoglobin, iron, transferrin, calcium, phosphorous, Cu, Zn, total and HDL cholesterol, triglycerides, alkaline phosphatase, ALT, AST, total Immunoglobulin, IgA and IgG antigliadin, and anti-Helicobacter pylori antibodies, and ferritin. Plasma folates, B12, and IgE also were determined, as were red blood cell volume, platelet, and eosinophil counts [8,11–16,19].

2.4.2. Anthropometry

Anthropometric measurements were obtained by standard techniques as described previously [11]. Children less than 2 years old were measured in length, while older children by height. Length and height were expressed as a percentage of median length (height) for age (NCHS, USA).

Weight was expressed as a percentage of the median weight for age (NCHS, USA). Weight for length/height was the individual weight divided by the median reference weight for the same height (NCHS, USA). Muscle area also was calculated [11].

2.4.3. Diary assessment

Seven-day home diaries, which reported hunger type recognition and glycemia measurements, were used to estimate energy, fiber, fruit and vegetable intake, environmental effective temperature, and document the hours spent outdoors, in gym, and asleep.

All data were recorded on special forms supplied by the investigators. Space was provided for reporting five meal events. Further occasional intake events were joined with the nearest meal. The meal was an event of energy intake sepa-
rated by more than 2 h from similar events in a day. A dietitian instructed each caretaker in food measurement and weighing. Meal initiation by either metabolic or external hunger was evaluated in the quarter of an hour before breakfast, lunch, and dinner, and was reported in the diary by the caregiver. The type of hunger feeling was recorded (stomach, mood and mind, physical). Food intakes were estimated by weighing or measuring foods before and after cooking. Measurement utensils were provided to the care-takers by the investigators and all portions after cooking and leftovers were weighed or measured. Glycemia was then measured for adults in a drop of total blood from a warm finger with an automated portable blood glucose meter (Miles). It was also often measured on willing children to better understand their expressions. Care was taken to avoid sampling cold fingers. The diary measurements made with the portable instrument were corrected by the ratio to the Hospital laboratory, and the same lot of strips was used in the individual diary succession (see Validations). The meter is based on measurement of the electrical potential produced by the reaction of glucose with the glucose oxidase reagents on the electrode. The capillary values are reported in this presentation, which are 0.2 mmol/L lower than the venous plasma glucose. The objective average compliance was calculated as the weekly mean of these glycemia measurements (DAP glycemia). Vegetable acceptance, which was the average percent of the recommended intake per meal in the examined week, was used in addition to vegetable weight to allow comparisons in subjects despite differences in age and type of vegetable intake.

Intake data were analyzed with a computer program containing the nutrient composition of 600 commonly consumed foods [28]. Additional details have been published previously [8,11–16,19].

The number of days with diarrhea, vomiting, abdominal pain, headache, and fever before enrollment was assessed retrospectively by interviewing caregivers at baseline. Records of these symptoms were kept daily using validated procedures for all subjects during the follow-up [8,11–16,19]. Records of school or work activity and of days off were kept for older children and adults.

2.4.4. Assessment of *H. pylori* infection

*H. pylori* IgG antibodies were assayed in the serum from frozen samples by using a commercial enzyme-linked immunosorbent assay (ELISA); Helori test, Eurosipital, Trieste, Italy [29]. Baseline and early frozen samples were available for all children. Concentration of the antibody was expressed in Units, which corresponded to a 1% point in a percentage index obtained by comparing the patient’s value with the positive control. Over 15 U in the antibody concentration was considered evidence of an *H. pylori* infection (see Validation). Serology, bacterium culture, the urea breath test, and histology have not been statistically different in establishing the diagnosis from Warthin–Starry staining of gastric biopsy specimens and the urease test in adults and children [30–34].

2.5. Validations

The cut point between positive and negative concentrations was investigated in 40 children undergoing upper gastrointestinal endoscopy. Three antral biopsy specimens were taken for histology, culture, and the urease test. A positive diagnosis of *H. pylori* infection was made if the bacterium was isolated or both a positive urease test and histological gastritis were found. A cut point of 15 U in the antibody concentration gave no false negatives and one false positive of 21 negative subjects.

Fresh whole blood was drawn into heparin tubes at each diary. One sample was centrifuged immediately and analyzed in duplicate by a laboratory hexokinase method (Hospital autoanalyzer Synchron CX7). The allowed spread for this measurement was 2% from average, measured in 50 regional laboratories every month. The second sample was tested with the portable instrument 5 or 10 times. Care was taken for a simultaneous measurement with the laboratory [35].

The variation coefficient (SD/mean) was 2.6% in the measurements with the portable instrument, and 6.0% in the ratio between these measurements and the Synchron CX7 in 80 diaries. The diary measurements made with the portable instrument were corrected according to this ratio to Synchron CX7. The comparison was repeated on another sample when the difference was higher than 10%. Twelve subjects were discarded as inaccurate, due to over 7% variation coefficients in the measurements with the portable instrument. The subject’s hunger and glycemia evaluation were obtained at the moment of blood sampling under intervention, and the mean difference between estimation and corrected measurement of blood glucose level had no significant difference from zero. The mean error (SD) in this estimation was 7.0 ± 3.8% of mean measured blood glucose in adults and 9.9 ± 5.3% in children (p < 0.05). Internal hunger was perceived in the range of 2.9 to 5 mmol/L.

Planning the emergence of metabolic hunger was taught mainly through phone calls. Subjects were taught how to evaluate energy and vegetable intake, environmental temperature, humidity, wind, clothing, hours outdoors, physical activity and type of work, number of hours before meal consumption, and the possibility of snacks. Energy intake and glycemia measurements were reported daily, and a logical trial and error search for the best planning was developed in the subsequent phone call. Interest and trustfulness were verified in these phone calls. Practitioners visited 47 homes during intervention and confirmed accurateness within 10% in reported item [8].

2.6. Statistical analyses

The DAP glycemia in a week and the serum concentration of antibody to *H. pylori* were investigated by factor analysis (principal components) and simple regression analyses. These were carried out on absolute values after joining the two groups in comparison in both studies at the follow-up end (cross-sectional analysis). The analyses also were
performed on the pre–post intervention differences in the “recovery” study (intra-individual, longitudinal analysis). Communality is the proportion of the variances of the analyzed variables, which is communal in factor analysis, and may suggest a communal underlying factor. In this investigation, the communality of \( H. pylori \) antibody concentration and DAP glycemia was investigated by longitudinal factor analysis. One variable was added to the analysis at a time in search of potential confounders, which may change the communality. A selection was made of variables that showed a correlation coefficient of 0.45 or higher to the communality without decreasing the proportion of communality (potential confounders or consequences). The fraction of the meals that were consumed after recognition of metabolic hunger was considered 0 at baseline, and absolute values under intervention were used in longitudinal analyses on intra-individual difference of DAP glycemia and concentration of anti-\( H. pylori \) serum antibody. Simple regression analyses were investigated between longitudinal differences and baseline on each anthropometric measure [25].

The size sample was calculated to obtain an approximate change of 2.0–2.5% in the mean and SD of results after entry of any further subject. Values are expressed as means ± SD. The two-tailed \( t \)-test was used to detect significance of difference and correlation, and the level of interest was set at \( p < 0.05 \) [25].

### 3. Results

The 5-month educational period was completed by 55–59% of patients in the general population until 1986, and by 84–90% of those who were thereafter addressed and prepared for the intervention by interpersonal relationships or their physician. The caregiver often missed the surveillance of one main meal per day, and a few subjects were motivated to drop out due to the inability to program other meals. Overprotection and unmotivated fear of insufficient eating and aversion to fruit and vegetables were widespread in the general population in 1982. Eleven of 78 subjects abandoned the follow-up in the first 5 months after this date. One could not be traced due to change of address. One refused vegetables, and nine compliant and grateful subjects were motivated to drop out due to well-being and lack of need for further verification. Control subjects also reported this motivation and showed a dropout rate similar to that of intervention subjects (Table 1). Relapses of symptoms were observed in approximately 10% of subjects under intervention, and were associated with transient lack of compliance with the waiting for hunger perception. Complication or untoward effect was not reported. A difficulty distinguishing hunger was observed in two adults. These subjects predicted glycemia with a 5–6% error, despite no threshold hunger sensation. Hypoglycemia or fainting was not observed in children, and was associated with avoidance of fiber intake in three adults.

No significant difference was found in anthropometric or blood parameters, in intake estimates at baseline, or in length of follow-up in the selected subgroups for “prevention study” (Table 2). A mean follow-up of approximately 8 years was obtained. Maintenance of compliance was checked every year, and yearly results were comparable to those observed at final assessment. Recognition of metabolic hunger was reported in 72.0% of meals. Preprandial glycemia was measured in 19 subjects under intervention and in only four controls. A significant 8.5% lower glycemia was observed in the diary averages under intervention than in controls. Estimated energy intake was lower in the intervention than in control group, but the significance of the difference was marginal, and disappeared after adjustment for body weight. A significantly lower increase was observed in the mean weight for height under intervention than in control subjects, and the final value was significantly lower under intervention. A significant difference in fruit and vegetable intake also was observed between intervention and control group (Table 3).

No \( H. pylori \) seroconversion was observed in those who were under intervention for 8 years. The frozen sample of one subject was transiently positive after 8 years and negative after 12 years. This transiently positive subject was recalled with the 12-year cohort, and he was reported only here, without further mention in the 8-year cohort. Seven

---

**Table 2**

<table>
<thead>
<tr>
<th>Group composition and characteristics in the “prevention” and “recovery studies”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Entry age (m)</td>
</tr>
<tr>
<td>Age at last measurement (m)</td>
</tr>
<tr>
<td>Average follow-up period (m)</td>
</tr>
<tr>
<td>Parents’ school years*</td>
</tr>
</tbody>
</table>

* Recognition of “metabolic hunger.”

* “External hunger,” i.e., ad lib. eating.

* \( m = \) months.

* Sum of school years divided by 2.
Table 3
Final outcomes of anthropometric measurements and average intakes and preprandial glycemia reported in home diaries for infants enrolled at the second year of age and negative for Hp (“prevention” study), and for subjects enrolled between the fifth and 25th year of age and positive for Hp (“recovery” study)

<table>
<thead>
<tr>
<th></th>
<th>“Prevention” (n = 43)</th>
<th></th>
<th>“Recovery” (n = 24)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.1 ± 3.5</td>
<td>10.1 ± 3.6</td>
<td>12.8 ± 5.5</td>
<td>13.7 ± 5.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>32.7 ± 13.5</td>
<td>34.4 ± 13.6</td>
<td>43.6 ± 19.5</td>
<td>46.1 ± 16.6</td>
</tr>
<tr>
<td>Weight for height (%)</td>
<td>96.1 ± 12.3</td>
<td>102.9 ± 13.0</td>
<td>103.2 ± 12.2</td>
<td>105.6 ± 19.0</td>
</tr>
<tr>
<td>Increase in % weight for height</td>
<td>+3.1 ± 12.5</td>
<td>+10.3 ± 13.7</td>
<td>−5.1 ± 10.2</td>
<td>−2.0 ± 11.2</td>
</tr>
<tr>
<td>Arm skinfold thickness (mm)</td>
<td>9.1 ± 4.3</td>
<td>10.0 ± 3.9</td>
<td>10.9 ± 4.9</td>
<td>12.3 ± 6.6</td>
</tr>
<tr>
<td>Increase in the sum of skinfold thickness</td>
<td>+7.0 ± 9.6</td>
<td>+10.4 ± 11.7</td>
<td>−5.1 ± 10.5</td>
<td>−2.9 ± 10.6</td>
</tr>
<tr>
<td>Daily energy intake (kcal)</td>
<td>1228 ± 419</td>
<td>1527 ± 478**</td>
<td>1267 ± 471</td>
<td>1485 ± 572</td>
</tr>
<tr>
<td>Daily energy intake per kg (kcal)</td>
<td>37.6 ± 16.1</td>
<td>44.4 ± 16.8</td>
<td>28.8 ± 14.4</td>
<td>32.2 ± 20.0</td>
</tr>
<tr>
<td>Daily vegetable intake (g/kg)</td>
<td>10.2 ± 7.9</td>
<td>2.3 ± 1.7**</td>
<td>11.8 ± 9.1</td>
<td>7.3 ± 6.7</td>
</tr>
<tr>
<td>Daily fruit intake (g/kg)</td>
<td>5.0 ± 3.6</td>
<td>3.1 ± 2.2**</td>
<td>6.4 ± 5.0</td>
<td>3.7 ± 3.0</td>
</tr>
<tr>
<td>Mean preprandial glycemia (mmol/L)</td>
<td>4.6 ± 0.6</td>
<td>5.0 ± 0.3*</td>
<td>4.4 ± 0.4</td>
<td>4.8 ± 0.3***</td>
</tr>
</tbody>
</table>

* p < 0.05, t-test; **p < 0.01, t-test; ***p < 0.001, t-test.

The children were enrolled in the trial between 1982 and 1994. The fractions represent the number of positive subjects of those recalled and tested. Frozen samples were available from previous examinations, and positive samples are mentioned and not reported in the table.

The mean of these four diary average preprandial glycemia (DAP glycemia) is similar to larger series of children of the same age under ad lib eating condition. Capillary values, which are 0.2 mmol/L lower than venous plasma glucose.

Table 4
“Prevention” study. Number of subjects with positive seroconversion of the tested subjects in the intervention and control groups after 4, 8, and 12 years.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 years</td>
<td>0/14*</td>
<td>4/13</td>
</tr>
<tr>
<td>8 years</td>
<td>0/13#</td>
<td>3/14</td>
</tr>
<tr>
<td>12 years</td>
<td>2/16</td>
<td>6/16</td>
</tr>
<tr>
<td>Total</td>
<td>2/43**</td>
<td>13/43</td>
</tr>
<tr>
<td>%</td>
<td>4.7%</td>
<td>30.2%</td>
</tr>
<tr>
<td>Cumulative follow-up</td>
<td>352#</td>
<td>346#</td>
</tr>
</tbody>
</table>

* The children were enrolled in the trial between 1982 and 1994. The fractions represent the number of positive subjects of those recalled and tested. Frozen samples were available from previous examinations, and positive samples are mentioned and not reported in the table.

# Conversions of tested subjects.

% Conversions of subjects followed in the control group.

# One child under intervention was positive after 8 and negative after 12 years for anti-H. pylori antibody, and he is included only in the 12-year group.

* The pre–post difference in the mean concentration of serum anti-H. pylori was significantly higher in the intervention than in the control group. Significant positive correlation was found between the serum concentration of anti-H. pylori and DAP glycemia in simple regression longitudinal analysis (Fig. 1). Three-quarters of the two variances were communal in longitudinal factor analysis, 0.87 being the correlation coefficient of each variable to the communality. The fruit, vegetable, fiber, and energy intake and outdoors hours were intervention components. A significant positive correlation was found between energy intake adjusted for body weight and DAP glycemia. Significant negative correlation was found between the diary average fruit intake adjusted for body weight and the serum concentration of anti-H. pylori in simple longitudinal regression analyses (r = 0.37; p < 0.05, and r = −0.43; p < 0.01, respectively). Both relations shared all their variance with about half of the communal variance of DAP glycemia and of anti-H. pylori in factor analysis. The search for confounding variables on this
communality showed no effect by age or other investigated variables. Instead, the ratio between LDL and HDL cholesterol and serum IgA concentration showed a high positive correlation coefficient to the same communality. Outdoor hours and vegetable, fruit, and fiber intake showed a negative correlation. No relation to the communality was found by the following variables: body weight; body weight percent for height and for age; skinfold thickness; arm and leg muscle area; number of days with vomiting, headache, abdominal pain or diarrhea in the 90 days preceding end of follow-up; and parents’ sum of the number of school years. The following daily averages obtained from the 7-day home diary also showed no relation: fraction of meals induced by metabolic hunger; carbohydrate, protein, and fat intake adjusted for energy intake; effective home temperature; and hours spent in bed.

None of the groups showed any deterioration in intermeal behavior or in intellectual or physical school or work performance. There was no decrease in hours spent outdoors, no increase in bedtime hours, and no clinical deterioration in blood samples. The mean height and calculated muscle areas remained around the normal median reference for the same age (NCHS, USA) in all four groups.

4. Discussion

Four metabolic feelings or conditions were distinguished: (1) satiety or indifference at the sight of food; (2) appetite or consumption of available food at perception of it; (3) bearable hunger feelings between 3.9 and 4.7 mmol/L glycemia, or search for food without activity disturbance; (4) unbearable or bulimic hunger under 3.6–3.3 mmol/L, which was associated with activity disturbance. The last two types emerged with threshold characteristics in most subjects. The bearable or transient perception of metabolic hunger represented a metabolic signal rather than the beginning of an unpleasant period. Slow glycemia decrease and mild hunger have been observed with high fiber intake [15,21–24], and fruit and vegetables were more than doubled in the meals under intervention compared to controls. On the other hand, the high fruit and vegetable intake in association with no metabolic hunger or glycemia over 5 mmol/L produced abdominal pain and/or diarrhea [12].

The reader may consider hunger feelings and intake decision a psychological event with psychological purposes and consequences, and may not know how to perceive metabolic hunger as it was described in this investigation. The explorative verification required a modest amount of responsible and intense mental work and important changes in eating behavior, including copious fruit and vegetable intake and glycemia measurements. This full understanding and achievement of emergence of metabolic hunger before 75% of the meals (as in present investigation) may be impossible for many readers. On the other hand “most people can follow the present intervention if properly informed” and “the intervention was less difficult than expected” were typical reports by the subjects in this intervention. The error in the prediction of glycemia by metabolic feelings was within 10% of the measured value in these subjects. The evaluation of metabolic feelings showed an independent association with a steadier and slightly lower glycemia in preliminary investigations on 7000 meals by stratified analysis [14–16]. The plasma glucose concentration represents a balance between in and out energy fluxes [3–7]. The prediction of the emergence of metabolic hunger within a tight glycemia range at the scheduled mealtimes required the evaluation of these fluxes and the manipulation of a few. The resting metabolic rate and energy fecal loss significantly decreased under intervention [15]. The physical activity and hours spent outdoors could be predicted. Fruit, vegetable, fiber, and energy intake could be manipulated [14–16]. The manipulated or predicted factors of energy balance acted on glycemia, which in turn affected the serum concentration of antibody to H. pylori. The effect on the outcome might, however, be independent from glycemia lowering. This is-

---

Table 5
“Recovery” study

<table>
<thead>
<tr>
<th>Group</th>
<th>Entry</th>
<th>End-trial</th>
<th>End-trial prevalence of infection (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>(n = 24)</td>
<td>48.7 ± 38.2</td>
<td>29.6 ± 42.9()</td>
</tr>
<tr>
<td>Control</td>
<td>(n = 23)</td>
<td>47.7 ± 40.8</td>
<td>56.5 ± 42.5()</td>
</tr>
</tbody>
</table>

Serum anti-

---

Fig. 1. Positive correlation between serum concentration of anti-

---
issue was investigated in the subjects of the “recovery” study by simple regression and factor analyses. A negative correlation to the serum concentration of antibody to *H. pylori* (outcome) was shown by adjusted fruit intake, and this finding has been already reported [36]. The variance of this relation was in common with the larger variance of the relation between DAP glycemia to the outcome. This coincidence supports an inhibition of infection by fruit mediated by the maintenance of a lower glycemia. The reproduction of these investigations may confirm the role of the components, as well as conclude the demonstration.

The intervention was associated with prevention of *H. pylori*-positive seroconversion and recovery from it in the two studies. Longitudinal simple regression analysis showed a highly significant, positive correlation between the concentration of serum anti-*H. pylori* and DAP glycemia in the “recovery” study. The odd ratios were 6.5 times lower for positive seroconversion in the “prevention” study, and 4.8 times higher for negative seroconversion in the “recovery” study under intervention than in “ad lib,” control subjects. Lower weight for height increase was observed more in the group under intervention than in the control group in the “prevention study,” and this may confirm an actual compliance in recognizing metabolic hunger in the long period.

A dependence on age of *H. pylori* seroconversion is well documented [29], and was confirmed by the “prevention” study. The groups in this study were well matched for age at entry and length of follow-up to avoid any effect by this confounding variable. The modest and insignificant difference in age between the groups in the “recovery study” showed no relation to the concentration of serum anti-*H. pylori* in regression and factor analyses.

The bacteria number on duodenal normal mucosa decreased in time after the previous meal in children and the variation was significantly larger on flat mucosa [19]. Absorption acceleration has been observed in association with lower glycemia [3, 36–38, 40–42]. This acceleration was the purpose of intervention, to decrease the immune stimulation by intestinal bacteria. Present findings confirm the expectation for the stomach. Seroconversion is currently used for diagnosis of *H. pylori* infection [29]. This method has not been statistically different from bacterium culture, the urea breath test, histology, or Warthin–Starry staining of gastric biopsy specimens, and the urease test in establishing the diagnosis in adults and children [30–34, 43]. Improvements in gastric emptying and/or acid secretion might inhibit *H. pylori* proliferation. These gastric functions increase with insulin sensitivity increase and glycemia decrease [36–42].

The cohorts showed 10–15% dropout rates during the first 5-month learning period. Reasons for this were reported by phone; compliant persons who were satisfied with their present health did not feel the need to make a further visit. The same reasons or lack of time and money explained further dropouts at annual verification, and the dropouts were similar in the intervention and control groups. This similarity supports lack of bias in the dropouts from either cohort. This high compliance was, however, obtained in a selected population with different problems than most of the population. The majority of the human population develops risks and organ deterioration associated with well-being and not paying attention to one’s own body or its changes. A flat small intestinal mucosa often has been observed in well-being celiac children during gluten feeding in our Unit. Vascular disease develops silently through years [9, 10]. Inflammatory bowel disease may show anatomical lesions well after symptom regression [44]. Also, the *H. pylori* infection was not predictable by symptoms in this investigation. On the contrary, a minority of human patients has been described with excessive perception of or attention to their own body feelings in association with scanty anatomical alteration [45]. The investigated subjects were selected from this minority. These subjects considered the assessment of metabolic hunger feelings at meals and of physical fitness in the interval as an easy guide for all life. *H. pylori* infection was prevented or cured in these attentive subjects, and insulin resistance also improved in a preliminary report [14]. For prevention purposes, an increase in attention to own body feelings may also be useful to the vast majority, which develops vascular and ovary risks in association with no complaints.

**Acknowledgment**

This work was supported by Local University grants for the years 1990–1999 and CNR grants for 1992 and 1994.

**References**


