Morphology and disaccharidase activity of small intestinal mucosa in post-weaning-induced malnourished rats and after realimentation

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**ABSTRACT**

**Background** The most common cause of failure to thrive in infants is malnutrition which causes histological and biochemical changes of small intestine. Studies on histology and enzyme activity of small intestinal mucosa were not much developed.

**Objective** To study about histology and disaccharidases activity of small intestinal mucosa in post-weaning-induced malnourished rats.

**Methods** We used Sprague-Dawley white rats as the subjects of this study with post test-control group design. The study was performed at the CentER for Research of Food and Nutrition Development from April 2003 to December 2004.

**Results** There were a decrease of body weight (hypotrophy normoblastic) and an increase of disaccharidase activity of small intestinal mucosa in post-weaning-induced malnourished rats. After realimentation, there were an increase of body weight, an improvement of the hypotrophy, and a decrease of the enzyme but they still cannot reach normal condition.

**Conclusion** In malnourished rats, there are decrease of body weight hypotrophy normoblastic and increase of disaccharidases activity of intestinal mucosa that are improved after realimentation [Paediatr Indones 2006;46:229-235].

**Keywords**: small intestinal mucosa, disaccharidase activity, post-weaning-induced malnutrition, realimentation.

Malnutrition occurring in children of 1 year of age may cause growth disorder, that can be seen in children of 5 years of age as stunted child. When growth disorder occurs in a girl and continues until grown up, she will be a malnourished woman. Malnourished mother will deliver low birth weight infants, as a result of intrauterine growth retardation. Low birth weight infants have higher risk of morbidity and mortality due to diarrhea 3-4 times more frequent than that of normal birth weight infants.

Malnutrition can be recovered by adequate and balanced nutrition. In malnourished infants, realimentation can be given by calculating energy required to achieve the ideal growth. Cook’s study proved that realimentation recovered histology and biochemistry changes of gastrointestinal tract. After that, study on morphology and biochemistry was not much developed, especially about small intestinal en-
zyme activity in malnutrition. Based on this background, the authors conducted a study on the morphology and disaccharidases activity of small intestinal mucosa in post-weaning-induced malnutrition and after realimentation, by using experimental animal.

**Methods**

This was an experimental study using white rats Sprague-Dawley with post test-control group, performed at Center for Research and Development of Food and Nutrition, Bogor, from April 2003 to December 2004. Forty male litters of 8 weeks old which born to 5 female rats weighing 250-300 grams, were fed with standard laboratory diet containing complete nutrition with total of food calorie of 378 calorie/100 gram of food.

To obtain post-weaning-induced malnourished rats group, malnutrition induction had been done by diet restriction, i.e. 50% of the control intake. Control rats group was given food ad libitum. This post-weaning-induced malnutrition started right after weaning, at the age of 3 weeks to 12 weeks old. Realimentation was done 8 weeks after the end of induction period, to rats at the age of 12 weeks to 20 weeks old. At realimentation period, one of control rats group continued to get food ad libitum; one of post-weaning-induced malnourished rats group was not realimented, but continued to have diet restriction; and one of post-weaning-induced malnourished rats was realimented, with food given ad libitum. According to Frederer formula, each group should contain a minimum of 5 rats; we used 8 rats per group in our study.

At the end of post-weaning-induced malnourished period, one of control rats group and one of malnourished rats group were sacrificed to obtain data of post-weaning-induced malnutrition. At the end of realimentation period one of post-weaning-induced malnourished rats group which realimented were sacrificed, one of post-weaning-induced malnourished rats which also not realimented, and one of control rats group were sacrificed to obtain data of post-weaning-induced malnutrition after realimentation.

Data including body weight, other physical parameters (length, weight/length ratio), serum albumin level, small intestinal parameters (weight, length, diameter, mucosa weight); were collected by Centre for Research and Development of Food and Nutrition, Bogor. The examination of histology of mucosa (mucosa thickness, villus height, cryptus depth, ratio villus/crypta, number of villus), was performed at Veterinology Research Unit, Bogor. Serum albumin level was measured at Department of Clinical Pathology, Medical School, University of Indonesia. The determination of protein content, DNA content, protein/DNA ratio, disaccharidase activity of small intestinal mucosa were performed at Department of Biochemistry and Biomolecular, Medical School, University of Indonesia.

Morphology of small intestinal mucosa was evaluated by morphometry. Disaccharidases activity was measured by modification of Dahlqvist method. Homogenation of small intestinal mucosa tissue which had been prepared in distilled water was incubated in suitable substrate, i.e. lactase, maltase, and sucrase to hydrolyse the disaccharide. Glucose which is formed was measured quantitatively using glucose peroxidase.

**Results**

It was found that post-weaning-induced malnourished rats had lower body weight, serum albumin level, physical parameters, and small intestinal parameters than those of controls (Table 1).

Body weight and other parameters of realimented post-weaning-induced malnourished rats were higher than those of non-realimented rats but lower than those of control rats (Table 2).

Four weeks after realimentation, body weight of realimented post-weaning-induced malnourished rats was higher than that of non realimented rats, but at the end of realimentation (20 weeks old) it could not reach body weight of control rats (Figure 1).

Serum albumin level of post-weaning-induced malnourished rats after realimentation was higher than that of post-weaning-induced malnourished rats before realimentation (Figure 2).

Small intestinal mucosa of post-weaning-induced malnourished rats was hypotrophy and normoplasy (Table 3). Hypotrophy was confirmed based on mucosa thickness, villus height, and cryptus depth which were lower than control. This finding was also confirmed by a decrease of protein content and protein/DNA ratio. Normoplasy was confirmed based on DNA
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**TABLE 1.** Body weight*, other physical parameters*, serum albumin level*, and small intestinal parameters* of post-weaning-induced malnourished rats (12 weeks old)

| Parameters                      | Control (n=8)  | Malnutrition (n=8) |
|---------------------------------|----------------|-------------------|
| Body weight (g)                 | 213.0±19.7     | 148.6±14.0a       |
| Serum albumin level (mg/dl)     | 4.56±0.28      | 4.19±0.12a        |
| Body length (cm)                | 20.1±1.0       | 17.9±0.9a         |
| Body weight/length (g/cm)       | 10.6±0.6       | 8.3±0.5a          |
| Chest circumstance (cm)         | 30.6±0.9       | 22.9±1.8a         |
| Small intestinal weight (g)     | 6.9±0.6        | 5.7±0.8a          |
| Small intestinal length (cm)    | 119.7±3.7      | 105.3±8.6a        |
| Small intestinal diameter (mm)  | 3.0±0.2        | 2.3±0.1a          |
| Small intestinal mucosa weight (mg) | 2431.7±363.7 | 1840.1±590.8s    |

* Presented as mean and standard deviation.

a t test significantly different (P<0.05) from control.

**TABLE 2.** Physical parameters*, small intestinal parameters* post-weaning-induced malnutrition after realimentation (20 weeks old)

| Parameters                      | Control (n=8)  | Malnutrition (n=8) | Realimentation (n=8) |
|---------------------------------|----------------|-------------------|----------------------|
| Body weight (g)                 | 355.7±17.2     | 200.3±1.6         | 293.1±21.5a,c        |
| Body length (cm)                | 22.7±0.5       | 20.2±0.5          | 21.9±0.7a,c          |
| Body weight/length (g/cm)       | 15.7±0.5       | 9.9±0.2           | 13.4±0.6a,c          |
| Chest circumstance (cm)         | 38.9±1.2       | 24.3±1.2          | 31.1±0.7a,c          |
| Small intestinal weight (g)     | 8.6±0.7        | 4.9±0.4           | 7.4±1.0a,c           |
| Small intestinal length (cm)    | 129.5±7.5      | 111.5±4.8         | 124.4±11.0a,c        |
| Small intestinal diameter (mm)  | 3.2±0.1        | 2.2±0.1           | 2.7±0.2a,c           |
| Small intestinal mucosa weight (mg) | 207.9±183.7   | 1935.3±308.1     | 2709.6±390.2a,c      |

* Presented as mean±standard deviation.

a t test significantly different (P<0.05) from control.

Figure 1. The increase of realimentation body weight post-weaning-induced malnutrition from 12 weeks to 20 weeks.
content of small intestinal mucosa that remained unchanged compared to control.

After realimentation, there was a recovery of small intestinal mucosa growth but it could not reach the growth of control rats (Table 4).

Disaccharidase specific activity of small intestinal mucosa of post-weaning-induced malnourished rats was higher than that of control (Table 5).

After realimentation, disaccharidase specific activity of post-weaning-induced malnourished rats was lower than that of non-realimented post-weaning-induced malnourished rats, but it was higher than that of control rats (Table 6).

### Table 3. Mucosa parameters*, protein content*, and small intestinal mucosa DNA* post-weaning-induced malnutrition after realimentation (20 weeks old)

| Parameters                | Control (n=8) | Malnutrition (n=8) | Realimentation (n=8) |
|---------------------------|---------------|--------------------|----------------------|
| Mucosa thickness (μm)     | 688.1±109.2   | 455.7±37.2         | 485.6±46.8           |
| Villus height (μm)        | 431.3±47.5    | 299.4±36.0         | 386.9±33.3           |
| Cryptus depth (μm)        | 183.1±26.5    | 110.6±16.4         | 153.8±10.3           |
| Ratio villus/cryptus      | 2.4±0.4       | 2.7±0.4            | 2.6±0.3              |
| Number of villus (per mm) | 9.1±0.8       | 9.5±0.3            | 9.3±0.7              |
| Protein content (mg)      | 610.0±85.5    | 435.6±139.7        | 773.4±97.7           |
| DNA content (mg)          | 44.9±18.7     | 50.0±11.7          | 48.6±16.8            |
| Ratio protein/DNA         | 15.7±6.3      | 8.6±1.3            | 16.1±2.2             |

* Presented as mean±standard deviation.

### Table 4. Mucosa parameters*, in post-weaning-induced malnutrition after realimentation (20 weeks old)

| Mucosa parameters | Control (n=8) | Malnutrition (n=8) | Realimentation (n=8) |
|-------------------|---------------|--------------------|----------------------|
| Mucosa thickness (μm) | 995.0±219.1   | 433.8±38.3         | 485.6±46.8           |
| Villus depth (μm)  | 451.0±88.9    | 303.8±54.4         | 386.9±33.3           |
| Cryptus depth (μm) | 198.5±53.1    | 93.8±15.1          | 153.8±10.3           |
| Ratio villus/cryptus | 2.3±0.3       | 3.3±0.8            | 2.6±0.3              |
| Number of villus (per mm) | 8.8±0.5       | 9.9±0.7            | 9.3±0.7              |
| Protein content (mg) | 936.6±50.5    | 484.8±74.3         | 773.4±97.7           |
| DNA content (mg)    | 39.7±10.7     | 44.0±16.8          | 48.6±16.8            |
| Ratio protein/DNA   | 25.0±6.1      | 12.3±4.0           | 16.1±2.2             |

* Presented as mean±standard deviation.

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**FIGURE 2.** Serum albumin level of post-weaning-induced malnutrition and that after realimentation.
Discussion

Malnourished rats which was defined as body weight equal or less than 70% from normal rats were used because most studies used malnutrition rats weighing around 40-70% from body weight control rats. There are various malnutrition induced techniques used in previous studies, i.e. uterine blood reduction, low protein diet, diet restriction, and expanded litter technique. In this study we used diet restriction.

This study found a decrease of body weight, a decrease of other physical parameters, small intestinal parameters, and serum albumin level in malnourished rats. After realimentation the increase of body weight was also parallel with the increase of other parameters.

Similar results were found by Butzner and Gall using rabbit as experimental animal. They used expanded litter technique. Realimentation at lactation period was able to increase body weight of rats, but it could not reach normal body weight.

In this study the increase of body weight of realimented rats at 17 weeks was more slowly than at 12-16 weeks. Possible mechanism is that pancreas function disorder started at that age because of malnutrition, resulting nutrient malabsorption, and finally causes more slowly increase of body weight. Another possible mechanism is the development of small intestinal mitochondria epithel anomali caused by malnutrition, resulting energy forming process disorder. This study found that there was a hypotrophy of small intestinal tissue in post-weaning-induced malnourished rats. Different findings was found by Butzner and Gall. They used rabbit as experiment animal and expanded litter technique as malnutrition induction method. They found protein content and DNA content reduction, it means that malnutrition affects small intestinal mucosa in size as well as in number of cells. Assuming that malnutrition induction using expanded litter technique has the same effect with diet restriction methods, the possibility of why the result was different was due to the difference of the experimental animals.

In post-weaning-induced malnourished rats after realimentation, there was an improvement of small intestinal hypotrophy, but it could not reach normotrophy. This study found that disaccharidases specific activity in post-weaning-induced malnourished rats was higher than that of control rats. Different results were reported by Jambunathan et al; they found no difference with that of control rats.

Lactase, maltase, and sucrase specific activities of post-weaning-induced malnourished rats, which were higher than control rats, were not parallel with the reduction of villus length. Perhaps there was intestinal adaptation mechanism in malnutrition.

In post-weaning-induced malnourished rats after realimentation there were reduction of disaccharidases activity, lactase, maltase, also disaccharidases, even though it could not reach the control value.

The findings of morphology and disaccharidases activity in post-weaning-induced malnourished rats and

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**Table 5. Disaccharidase Specific Activity* in Post-Weaning-Induced Malnourished Rats (12 Weeks Old)**

| Parameters | Control (n=8) | Malnutrition (n=8) |
|------------|--------------|------------------|
| Lactase**  | 34.7±5.3     | 92.8±23.3*       |
| Maltase**  | 609.9±33.6   | 997.0±111.5*     |
| Sucrase**  | 53.9±6.7     | 109.4±23.2*      |

*Presented as mean±standard deviation.
**Activity in ìmol/menit/g mucosa.
* t test significantly different (P<0.05) from control.

**Table 6. Disaccharidase Specific Activity* in Post-Weaning-Induced Malnourished Rats after Realimentation (20 Weeks Old)**

| Parameters | Control (n=8) | Malnutrition (n=8) | Realimentation (n=8) |
|-----------|--------------|--------------------|---------------------|
| Lactase** | 36.3±11.7    | 102.3±18.7         | 65.9±8.1*          |
| Maltase** | 584.1±61.5   | 1027.9±181.3       | 732.5±68.4*        |
|Sucrase**  | 52.6±3.0     | 110.1±31.3         | 76.0±8.0*          |

*Presented as mean±standard deviation.
**Activity in ìmol/menit/g mucosa.
* t test significantly different (P<0.05) from control.
* t test significantly different (P<0.05) from malnutrition.
after realimentation, provide contribution to malnutrition prevention effort by giving realimentation for children to prevent small intestinal mucosa morphology and biochemistry disorders caused by malnutrition.

We concluded that in malnourished rats, there is a reduction of body weight, small intestinal mucosa hypotrophy normoplastic, and an increase of disaccharidases activity. In post-weaning-induced malnourished rats after realimentation, there is an increase of body weight but it cannot reach normal, an improvement of small intestinal mucosa hypotrophy but it cannot reach normotrophy, and a reduction of disaccharidases activity but it cannot reach normal value. It is assumed that realimentation is able to improve small intestinal mucosa growth optimally in post-weaning-induced malnourished rats.

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