PRELIMINARY REPORT

Higher Body Fat Aggravates Toxin-Induced Infectious Episodes

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Animal models using rabbits were developed to accumulate a variable body fat mass (FM) in two groups of animals while the fat-free mass (FFM), eg, total body protein, was maintained essentially similar between the groups. Thiobarbituric acid–reactive substances (TBARS) were measured as an index of lipid peroxidation and were found to be higher in the whole-body tissues of animals with a higher FM. Bacterial toxin (lipopolysaccharide [LPS]) also induced higher lipid peroxidation in animals with a higher FM, with a concomitant incidence of bloody mucous diarrhea. To our knowledge, this is the first evidence to show the effect of body FM to aggravate toxin-induced infections leading to diarrhea. The overall results suggest further investigations to explore the possible role of body fat in infectious diseases in humans.

THE HUMAN BODY is mainly composed of a fat mass (FM) and a fat-free mass (FFM), of which the FFM contains total body water, minerals, solid bone mass, and total body protein. Protein-energy malnutrition (PEM), characterized by deterioration or alteration of the FFM, is associated with many infectious diseases, eg, human immunodeficiency virus infection leading to acquired immune deficiency syndrome. Chandra et al[1] have shown a concurrence of visceral protein synthesis and immunocompetence with an alteration of the FFM. They also concluded that PEM, as reflected by the FFM, is strongly associated with a high risk of morbidity and mortality. Body protein has the greatest impact on the production of endogenous mediators such as macrophages, neutrophils, and cytokines. A reduced activity of these mediators renders a PEM subject highly susceptible to various opportunistic diseases.

The other body compartment, ie, the FM, when excessively large as in obesity, contributes significantly to the development of life-threatening diseases such as diabetes, cancer, and cardiovascular disease.[2] It has been reported that cytokines, eg, tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1), are overexpressed in adipose tissue, which may initiate lipid peroxidation in this environment.[3,4] Lipid peroxidation, while associated with obesity-related diseases, is also implicated in many infectious episodes.[5] Although associations between the FM and infectious diseases, as in PEM, are limited in the literature, a few reports showed significant correlations for obesity with several infectious episodes, including diarrhea.[6,7]

Infections can be simulated by a challenge with endotoxins in an animal model. This study was therefore designed to investigate the role of the FM in infection using rabbits. Dietary manipulations were made to accumulate an excessive FM in one group of animals, while the FFM or protein mass remained necessarily similar in both groups. The results show that a higher body fat content enhanced toxin-induced lipid peroxidation, leading to bloody mucous diarrhea.

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MATERIALS AND METHODS

A total of 32 New Zealand white rabbits (both sexes included) were used in the study. At the age of 6 weeks, the animals were divided into two groups, A and B, each with an equal number of male and female rabbits. The mean body weight of groups A and B was 518 and 522 g, respectively. Animals in group A were offered a diet of 50 g/d containing an energy level of 4.41 cal/g (16% protein and 4% fat). Animals in group B received only half the amount of diet that was consumed on the previous day by group A. Group B animals were therefore designated as energy-restricted (ER) and group A animals as ad libitum (ad lib). The rabbits were kept in wire cubicles in an animal house where appropriate facilities for animal nursing exist.

The above-mentioned feeding practices were performed for 42 days, ie, for another 6 weeks. During this time, body weight and rectal temperature were monitored for each animal daily. At the end of 6 weeks, animals in each group (A and B) were separated into two subgroups (A1, A2, B1, and B2), with each pair, A1 and A2 or B1 and B2, containing eight rabbits matched by sex and body weight. For body composition measurements, animals in subgroup A1 and subgroup B1 were anesthetized with an appropriate dose of pentobarbital. Approximately 2-mL blood samples were collected from each animal for the determination of lipid peroxidation. The animals were killed with an overdose of pentobarbital, shaved, and then weighed. Carcass analyses for the assessment of FFM in terms of protein mass and FM were performed with the usual chemical-extraction methods.[8] Thiobarbituric acid–reactive substances (TBARS) were measured as an index of lipid peroxidation using spectroscopic methods already set up in our laboratories.[9] Aliquots of both blood and whole-body tissue (minced-meat) samples were used for TBARS measurement.

The remaining rabbits, eight in group A2 (ad lib) and eight in group B2 (ER), were challenged with lipopolysaccharide (LPS), at a dose of 4 mg/kg administered through an ear vein. After 24 hours, the rectal temperature was recorded and the feces were examined for consistency, volume, and appearance. Approximately 2-mL blood samples were withdrawn from each animal for assessment of TBARS as already described.

RESULTS

Changes in body weight in the rabbits after 42 days are listed in Table 1 as a function of the feeding practice, ie, Ad lib and ER. The weight increase was significantly higher in Ad lib animals than in ER animals (1,291 *v* 874 g). Carcass analyses showed that the amount of protein mass between the two groups was not different, while the FM was significantly higher in Ad lib animals versus ER animals (6.01 *v* 2.02 g%). This means that the higher body weight of Ad lib animals was mainly due to the accumulated fat weight. While the blood TBARS values were not different between the two groups (0.62 ± 0.06 *v* 0.59 ± 0.06

Table 1. Physiological Parameters of the Rabbits

Parameter	Group A1 (Ad lib, n = 8)	Group B1 (ER, n = 8)	P*
Age (wk)	12.2 ± 0.3	12.3 ± 0.3	NS
Body weight (g)			
Initial	518.0 ± 58.0	522 ± 60.0	NS
Final	1,291.0 ± 130.0	874.0 ± 92.0	<.001
Rectal temperature (°C)	37.3 ± 0.6	36.6 ± 0.5	<.024
Total body protein (g%)	17.20 ± 0.90	17.72 ± 1.23	NS
TBARS (μmol)			
Blood (per liter)	0.62 ± 0.06	0.59 ± 0.06	NS
Body tissue (per gram)	3.74 ± 3.12	0.91 ± 0.34	<.023

*Student's *t* test.

μmol/L), TBARS values were significantly different in the whole-body tissues of Ad lib and ER animals (3.74 ± 3.12 *v* 0.91 ± 0.34 μmol/g) (Table 1). This perhaps supports the notion that oxygen, being lipophilic, is capable of inducing more lipid peroxidation in a lipid milieu.[10] Higher lipid peroxidation in the body of the Ad lib animals may be responsible for the higher rectal body temperature.

Table 2 shows physiological and morbidity data for the other subgroups (A2 and B2) of animals that were challenged with LPS (bacterial toxin). As expected, these animals have a similar body weight gain versus animals in subgroups A1 and B1 (Table 1). Rectal temperature was increased in both groups of animals examined 24 hours after administration of LPS. Blood TBARS were also elevated in both groups compared with the normal animals, ie, those not challenged with LPS. However, blood TBARS were significantly higher in the Ad lib group versus the ER group (Table 2). Concomitantly, the Ad lib group had bloody mucous diarrhea, while the ER group under similar conditions did not show any apparent effects of LPS, except that they became somewhat less active once challenged with LPS.

DISCUSSION

The ER animals accumulated a small FM (2.02 g%) compared with the Ad lib animals (6.01 g%, $P < .001$), while the protein mass in both groups was essentially similar. The increased body weight in Ad lib animals was therefore mainly due to the accumulated FM. The changes in body composition in rabbits in this study agree very well with a recent human study that reported a significant loss of body FM on short-term dietary energy restriction.[11]

Table 2. Physiological and Morbidity Parameters of Rabbits Injected With LPS

Parameter	Group A2 (Ad lib, n = 8)	Group B2 (ER, n = 8)	P*
Age (wk)	12.2 ± 0.3	12.3 ± 0.3	NS
Body weight (g)			
Initial	518 ± 58.0	522 ± 60.0	NS
Final	1,293 ± 131.0†	873.0 ± 59.0†	<.001
Rectal temperature (°C)	38.8 ± 0.5	38.1 ± 0.5	NS
Blood TBARS (μmol)			
Normal	0.62 ± 0.06	0.59 ± 0.06	NS
LPS	1.73 ± 0.22‡	1.27 ± 0.11‡	< 001
Diarrhea	Bloody mucus	None	

*Student's *t* test.
†$P < .001$, initial *v* final.
‡$P < .001$, normal *v* LPS.

It is generally accepted that oxygen is lipophilic, which may facilitate peroxidation of lipid molecules in this environment.[10] This notion is well supported by the TBARS values in whole-body tissues of Ad lib animals with a higher FM (3.74 μmol/g) compared with the ER animals (0.91 μmol/g, $P < .025$), although TBARS values in blood samples did not differ significantly between the groups (0.62 *v* 0.59 μmol/L). Conversely, however, blood TBARS values in LPS-challenged animals increased significantly in both groups (Table 2), and this increment, once again, is significantly higher in Ad lib animals. Increased TBARS indicate the increased lipid peroxidation that accompanied the higher body temperature observed in these animals.

Lipid hydroperoxides formed due to lipid peroxidation are well known to affect cell membranes, thus resulting in an aberration of cellular metabolism, particularly ion transport.[12] This situation could be observed in the Ad lib animals in this study, who had LPS-induced bloody mucous diarrhea. LPS has been shown to invoke inflammatory mediators such as neutrophils, macrophages, and particularly cytokines.[13] Therefore, a toxin-induced increase in free-radical activity is expected to enhance lipid peroxidation, as observed in the blood of LPS-challenged animals in this study. Lipid hydroperoxides could also be formed from arachidonic acid by 5-lipoxygenase during lipid peroxidation.[14] Arachidonic acid is also the precursor of prostaglandins, which are capable of producing diarrhea.[15] Based on these reports, and assuming the LPS-induced inflammatory activity to be similar in both Ad lib and ER animals with a similar FFM, the observation of higher lipid peroxidation followed by bloody mucous diarrhea may therefore be attributed to the higher FM in the Ad lib animals. Cytokines, particularly TNFα and IL-1 as mentioned earlier, are expressed in adipose tissue,[3] ie, in the FM. These cytokines are also known to be involved in many infectious episodes. In fact, recently, our group[16] and others[17] have found a significant enhancement of TNFα due to infection with *Helicobacter pylori*, a gram-negative bacterium, in humans with a higher body mass index (BMI). The BMI is a qualitative measure of body fat. The findings of this preliminary study therefore suggest prospective investigations to further explore the role of body fat in infectious diseases in human subjects.

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