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Influence of Dietary Vitamin A and Iron Deficiency on Hematologic Parameters and Body Weight of Young Male Wistar Rats

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Micronutrient deficiency is one of the most prominent public health concerns; in particular, vitamin A and iron are determinants of appropriate development, and vitamin A influences iron homeostasis and metabolism. Here we compared the effects of diets that were sufficient and insufficient in vitamin A and iron on the hematologic parameters and body weight of rats. Male Wistar rats were randomly divided into 5 dietary groups (n = 7 per group): adequate in iron and vitamin A (control); adequate in iron but low in vitamin A (FesvAi); adequate in iron but lacking vitamin A (FesvAd); low in iron but adequate in vitamin A (FeivAs); and low in both iron and vitamin A (FeivAi). After 6 wk, rats showed significant differences in serum iron relative to the control diet (control, 256 ± 44 µg/dL; FesvAi, 220± 16 µg/dL; FesvAd, 181 ± 15 µg/dL; FeivAs, 131 ± 44 µg/dL; FeivAi, 75 ± 19 µg/dL). Rats on iron-deficient diets showed reduced Hgb values relative to the control diet (control, 15.9 ± 0.7 g/dL; FeivAs, 13.2 ± 1.6 g/dL; FeivAi, 12.9 ± 1.0 g/dL), MCV (control: 57 ± 10 fL; FeivAs, 48 ± 10 fL; FeivAi, 44 ± 3 fL), and Hct (control, 53% ± 2%; FeivAs, 44% ± 5%; FeivAi, 42% ± 8%). All of the experimental dietary groups showed significant differences in reticulocyte count when compared with the control group (control, 2.7% ± 2.2%; FesvAd, 0.6% ± 0.2%; FesvAi, 0.3% ± 0.1%; FeivAs, 1.2% ± 0.2%; FeivAi, 0.6% ± 0.5%). The mean difference in body weight for the experimental groups, relative to the control group, was 30 ± 10 g. These results suggested that, in young male Wistar rats, both iron and vitamin A are essential to cause increases in body weight and various hematologic parameters.

Abbreviations: ID, iron deficiency; VAD, vitamin A deficiency

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Micronutrient deficiency is a major public health concern in developing countries.[9] Vitamin A deficiency (VAD) and iron deficiency (ID) anemia are determinants for the cognitive, physical, and behavioral development of children.[40,43] Iron is an essential mineral that belongs to the group of transition metals; because of its chemical and physical properties, iron plays a major role in the metabolisms of most living beings. Iron is found in numerous proteins, particularly those associated with oxygen transport. Therefore, in practical terms, for many organisms, life would not be possible without iron.[32]

Anemia is a health condition that is linked to an inadequate supply of oxygen to the tissues. In children and young adults, anemia is expressed as reduced cognitive performance,[35] inadequate physical development, and immune system problems;[25] therefore, anemia is a high-impact public health matter in developing countries. Approximately 42.6% of preschool-age children are anemic, as are is 38.2% of pregnant women and 29.0% of nonpregnant women. Thus, overall, 496.3 million people are anemic.[45]

Often anemia is linked only to ID; however, although ID is responsible for 35% of anemia cases, there are other causes—both nutritional and otherwise. The nutritional causes include other micronutrient deficiencies and antinutritional factors, and other common causes are bleeding, infection, and genetic conditions.[27] ID anemia occurs when ferritin levels are below 12 µg/L and when Hgb levels are below established cutoff points (11 g/dL for children younger than 5 y and pregnant women; 12 g/dL for children 5 to 12 y old, nonpregnant women, and teenage males; and 13 g/dL for teenage females).[17] The main causes of ID anemia are nutritional and antinutritional dietary factors that block the body's absorption or use of iron.[29,39]

Sources of dietary iron include meat (which provides heme iron), vegetables, and legumes (both of which provide nonheme iron). The absorption of most dietary iron occurs in the duodenum and proximal jejunum; the exact location depends on the physical state of the iron atom. Heme iron is strongly bound to globular proteins that must be hydrolyzed through gastric pH and both gastric and intestinal proteases. [18]

Nonheme iron requires gastric pH to promote its solubilization and release from natural components.[18] Both heme and nonheme iron are absorbed in the duodenum;[42,] however, the enterocyte's absorption mechanisms depend on the dietary source. Nonheme iron mainly starts in the ferric form (Fe^{3+}) and must be reduced to the ferrous form (Fe^{2+}). The duodenal cytochrome B mediates this process, and ascorbic acid (vitamin C) enhances it; once reduced, the iron is taken into the enterocyte through apical-side membrane proteins, divalent metal transporter 1 and heme carrier protein 1, which is specific to that form of iron.[10] In addition, heme carrier protein 1 takes in heme iron on the other side. Then, the intact heme iron nucleus is sent to an endosome, where a heme-oxygenase enzyme degrades it by releasing iron from the protoporphyrin. Finally, in the endosome, an isoform of divalent metal transporter 1 releases the iron into the cytosol.

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Once in the cytosol, heme and nonheme iron are indistinguishable.[3] However, in the enterocyte, dietary iron can be (1) included in the membrane mitochondrial cytochromes, where it contributes to the oxide-reduction processes that are necessary for the electron-transport chain; (2) sent to a transitory pool of free intracellular iron that heavily autoregulates iron intake; (3) stored in cellular ferritin protein; or (4) exported to the bloodstream through ferroportin, a membrane protein on the basolateral side of the enterocyte. Once outside of the enterocyte, a ferroxidase is needed to oxidize the iron back into the ferric form; this process can involve a membrane protein called hephaestin or a plasmatic protein called ceruloplasmin. After this process, the iron is bound to transferrin—the protein that transports iron to the various tissues that recognize it (through the membrane's transferrin receptors); nontransferrin-bound iron has also been identified in the bloodstream.[28]

VAD is the other critically important micronutrient deficiency declared by the WHO; vitamin A is a determinant of many metabolic processes, including sight, hematopoiesis, embryonic development, cell differentiation, various immune-system functions, and genic transcription.[44] Vitamin A is necessary to the production of rhodopsin, which is required for low-light vision, normal growth and development, reproduction, and maintenance of skeletal and epithelial tissues. Vitamin A includes retinol and other molecules that mimic retinol's biologic activity; all of these molecules are from the provitamin A carotenoid group.[36]

Some researchers have suggested that vitamin A plays a role in the metabolism of iron; for this reason, many countries have developed supplementation strategies for both iron and vitamin A to reduce the prevalence of anemia.[41] Although the exact interaction of iron and vitamin A is not well known,[23,40] several mechanisms have been suggested, including the regulation of either hematopoiesis[19,24] or various proteins related to iron homeostasis (for example, transferrin,[26] iron regulatory protein 2,[23] and hepcidin).[6,13,15]

We conducted this study based on the hypothesis that both vitamin A and iron must be taken together to ensure adequate weight gain and hematologic parameters. The objective of this study was, by using on 5 combinations of iron and vitamin A concentrations, to determinate the influences of vitamin A and iron on body weight and various hematologic parameters in young male Wistar rats (*Rattus norvegicus*).

Materials and Methods

Animals and diets. We conducted this study at the Bioterium of the Research Laboratories of Antioquia University (Medellín, Colombia). The sample comprised 35 male, SPF, Wistar rats (age, 21 d; weight, 62.5 ± 6.1 g; Neurosciences Laboratory, Antioquia University). We housed the rats, which had a mean body weight, in polycarbonate cages with stainless-steel wire covers. The environmental conditions involved a 12:12-h light:dark cycle, room temperature of 22 ± 2 °C, and relative humidity between 55% and 65%; the air was renewed every 4 min. The rats had free access to food and demineralized water from polycarbonate dispensers, and we weighed them every 2 wk by using a digital balance (resolution, 0.1 g; model BJ2100D, (Precisa, Dietikon, Switzerland). The Ethical Committees of Antioquia University (protocol no. 108.090217) and La Sabana University (protocol no. 55.170516) approved this study's procedures.

We randomly assigned the rats to 5 diets, with 7 animals per group; in these diets, iron was in the form of ferric citrate, and vitamin A was in the form of retinol acetate. We fed the control group an AIN-93G diet for growing rodents, which we modified to provide sufficient iron (45 mg/kg) and vitamin A (1200 µg/kg). We fed the experimental groups AIN-93G diets, which we modified dies as follows: FesVAd, 45 mg/kg iron and no vitamin A; FesVAi, 45 mg/kg iron and 120 µg/kg vitamin A; FeiVAs, 15 mg/kg iron and 1200 µg/kg vitamin A; and FeiVAi, 15 mg/kg iron and 120 µg/kg vitamin). For each group's cage, we measured food intake weekly during the experimental period by weighing the remaining food in the cages, then divided that total intake by the number of rats in the cage to get the food intake per animal. Table 1 shows the composition and nutritional profile of each diet; all diets were produced by Research Diets (New Brunswick, NJ).

After 6 wk of feeding, we anesthetized the rats by using isoflurane (Piramal Critical Care, Mumbai, India) and used cardiac punctures to collect 500 µL of whole blood in microtainer tubes with EDTA-K2 (Impromini, Improve Medical Instruments, Guangzhou, China) for later analysis of the hematologic parameters. We also collected 2 mL in serum tubes (Vacutest gel with a cloth activator, Vacutest Kima, Arzegrande, Italy) to analyze the iron and retinol levels. After extracting their blood, we euthanized the animals with 70% CO_2. We preserved the liver, spleen, and small intestine of each rat for further analysis.

Biochemical parameters. Using centrifugation (448 to 700 × g/min for 15 to 20 min), we separated the fraction of blood that we had kept for iron and retinol analysis after complete clotting. We divided the supernatant into 2 fractions to determine serum iron and serum retinol levels. We analyzed the serum iron concentrations by using atomic absorption spectrometry (Analyst 3100 Analyzer, Perkin Elmer Life Sciences, Wellesley, MA) and measured the serum retinol concentrations by using a HPLC–UV apparatus (model PU-2080 plus chromatography pump, UV-2075 UV detector, and 807-IT integrator; Jasco, Tokyo, Japan).

Hematologic parameters. We measured the hematologic parameters—including Hgb, MCV, RBC count, reticulocyte count, Hct, MCH, and MCHC—by using an automated hematology analyzer (model URIT-2900, Urit Medical, Guangxi, China).

Statistical analysis. We analyzed the data by using SPSS software (SPSS Statistics for Windows, version 25.0, IBM, Armonk, NY) and examined the data for normal distribution by using the Kolmogorov–Smirnov test. We expressed the data as mean ± 1 SD and then analyzed the mean differences between the control and experimental groups nu using the Least Significant Difference test. We analyzed weight gain by using repeated-measures ANOVA. We considered P values of less than 0.05 to be significant. According to a power analysis, using 7 rats in each group achieves 80% power at a confidence level of 95%; previous researchers obtained a difference of 4 g/dL of Hgb content according to a SD of 2.39 g/dL.[12]

Results

Biochemical parameters. All of the intergroup comparisons indicated significant differences in serum iron content among male rats. Both groups with low amounts of dietary iron (FeivAs and FeivAi) had significant differences from the control group ($P < 0.001$ for both groups). VAD (FesvAi and FesvAd) altered the total serum iron content ($P = 0.024$ and 0.001, respectively), and the FesvAd group exhibited the lowest values.

In terms of serum retinol levels, the groups with insufficient or no dietary vitamin A all had significantly lower levels of serum retinol than the control group ($P < 0.001$ for both groups). Among the multiple comparisons, only the rats given diets with low vitamin A (FesvAi and FeivAi) showed no significant intergroup difference ($P = 0.980$; Table 2)

Table 1. Ingredient composition and nutritional profile for each diet

Ingredient	Control	FesvAd	FesvAi	FeivAs	FeivAi
Corn starch	397.5	397.5	397.5	397.5	397.5
Casein	200.0	200.0	200.0	200.0	200.0
Maltodextrin 10	132.0	132.0	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0	100.0	100.0
Soybean oil	70.0	0	0	0	0
Cotton Seed Oil	0	70.0	70.0	70.0	70.0
Cellulose	50.0	50.0	50.0	50.0	50.0
Mineral mix[a]	35.0	35.0	35.0	0	0
Mineral mix[b]	0	0	0	35	35
Vitamin mix[c]	10.0	0	0	10.0	0
Vitamin mix[d]	0	10	10	0	10
L-Cysteine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Ferric citrate [17.4% Fe]	0.03	0.03	0.03	0.07	0.07
t-Butylhydroquinone	0.014	0.014	0.014	0.014	0.014
Vitamin A palmitate [500.000 IU/g]	0	0	0.0008	0	0.0008
Protein [%]	20	20	20	20	20
Fat [%]	7	7	7	7	7
Fiber [%]	5	5	5	5	5
Carbohydrate [%]	64	64	64	64	64
Iron [mg/kg]	45	45	45	15	15
Vitamin A [µg/kg] as retinol acetate	1200	0	120	1200	120

d, deficient; i, insufficient; s, sufficient
Ingredients are listed as g/kg except when noted.
[a]AIN-93G-MX.
[b]AIN-93G-MX deficient in iron.
[c]AIN-93G-VX.
[d]AIN-93G-VX without vitamin A

Table 2. Biochemical parameters.

Parameter	Control	FesvAd	FesvAi	FeivAs	FeivAi
Serum iron [µg/dL]	256 ± 44[a]	181 ± 15[b]	220 ± 16[c]	131 ± 44[d]	75 ± 18e
Serum retinol [µmol/L]	1.60 ± 0.16[a]	0.24 ± 0.04[b]	0.55 ± 0.10[c]	1.26 ± 0.12[d]	0.65 ± 0.08[c]

$n = 7$ rats per group; values with different superscripted letters differ significantly ($P < 0.05$).

Hematologic parameters. Table 3 summarizes the results regarding hematologic parameters for the 5 groups. The groups with low dietary iron (FeivAs and FeivAi) had decreased levels of Hgb ($P < 0.001$ for both groups), MCV ($P = 0.002$ and 0.041 respectively), and Hct ($P = 0.007$ and 0.002 respectively) than the control group had. All of the experimental groups showed significant differences in reticulocyte count when compared with the control group (FeivAi, $P < 0.001$; FeivAs, $P = 0.010$; FesvAi, $P = 0.001$; FesvAd, $P < 0.001$). For MCH, only the FeiVai group was significantly ($P = 0.006$) different from the control group. There were no significant intergroup differences in RBC count or MCHC.

Body weight and food intake. Body weight at baseline was comparable among the 5 groups of rats (Figure 1) By week 5, rats fed the FesVai diet weighed significantly less than the control group ($P = 0.005$). After week 7, all experimental groups showed significantly less weight gain than the control group (FeivAi, $P < 0.001$; FeivAs, $P = 0.030$; FesvAi, $P < 0.001$; FesvAd, $P = 0.006$). The comparisons among the experimental groups after week 7 revealed no significant differences between them. At the end of the experiment, the mean difference in body weight for the experimental groups, relative to the control group, was 29.8 ± 10.0 g. The groups showed no significant differences in food intake ($P = 0.126$); the average daily food intake per rat was 15.5 g/d, which is consistent with the expected food intake for growing animals of this type.[33]

Discussion

Among the groups of rats fed diets containing differing amounts of iron and vitamin D, all of the intergroup differences in serum iron were significant, running from highest to lowest as: Control > FesvAi > FesvAd > FeivAs > FeivAi. The first 3 groups in this list had sufficient iron, with decreasing vitamin A from the first through the third groups; our finding suggests that, even with an adequate amount of iron in the diet, insufficient vitamin A can cause serum iron levels to decrease proportionally. These results are consistent with previous findings[40] regarding a positive association between serum retinol deficiency and serum ID in people from Vitoria, Brazil. Other authors have similarly linked VAD with ID across many epidemiologic studies. For example, the odds anemia in children

Table 3. Hematologic parameters

Parameter	Reference value	Group				
		Control	FesvAd	FesvAi	FeivAs	FeivAi
Hgb [g/dL]	15.2 ± 1.3	15.9 ± 0.7[a]	16.2 ± 2.4[a]	15.6 ± 0.4[a]	13.2 ± 1.6[b]	12.9 ± 1.0[b]
MCV [fL]	62.4 ± 1.4	56.8 ± 9.9[a]	59.9 ± 2.3[a]	58.2 ± 6.9[a]	48.3 ± 9.6[b]	43.6 ± 3.3 [b]
RBC count [×10^6/μL]	8.2 ± 0.3	9.5 ± 1.3[a]	8.9 ± 0.9[a]	9.0 ± 1.6[a]	8.9 ± 2.0[a]	10.0 ± 0.9 [a]
Reticulocyte count [%]	2.0 ± 1.0	2.7 ± 2.2[a]	0.6 ± 0.2[b]	0.3 ± 0.1[b]	1.2 ± 0.2[b]	0.6 ± 0.5[b]
Hct [%]	51.3 ± 1.4	52.9 ± 2.1[a]	52.9 ± 4.3[a]	51.4 ± 6.3[a]	43.7 ± 4.7[b]	42.2 ± 8.1[b]
MCH [pg]	18.5 ± 1.5	17.2 ± 3.5[a]	18.3 ± 1.8[a]	17.9 ± 2.9[a]	15.2 ± 2.9[a]	13.0 ± 1.1[b]
MCHC [g/dL]	29.7 ± 2.2	30.2 ± 1.6[a]	30.5 ± 2.5[a]	30.7 ± 3.5[a]	29.8 ± 2.0[a]	31.7 ± 3.5[a]

n = 7 rats per group. Reference values are according to reference 4 except for reticulocyte count, which is according to reference 22. Data are given as mean ± 1 SD; values with different superscripted letters differ significantly ($P < 0.05$).

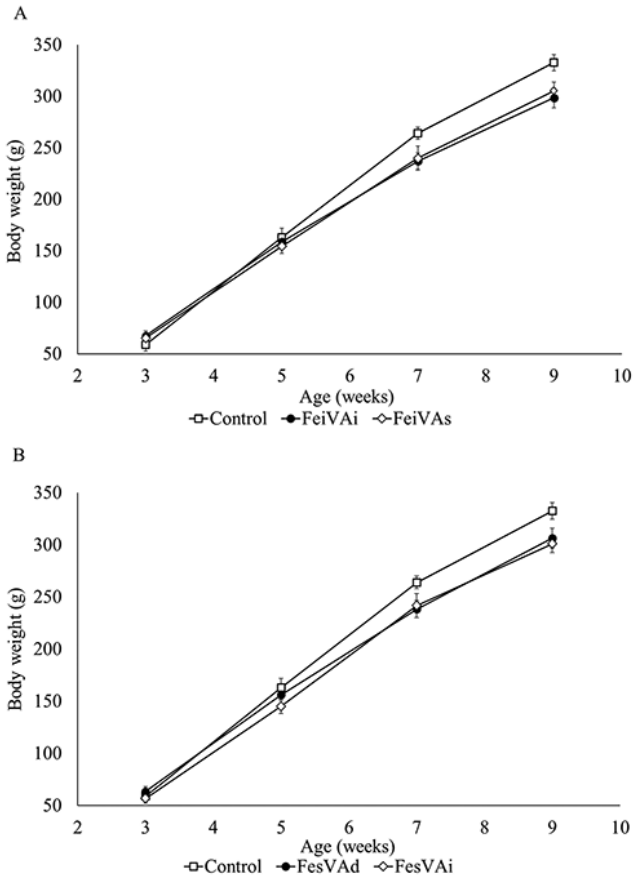

Figure 1. Growth curves of the (A) iron-sufficient and (B) iron-insufficient diets, as compared with the control diet. Data are given as means ± 1 SD (error bars).

with VAD are 2.5 times higher (odds ratio = 2.5; 95% CI, 0.027 to 0.049; $P < 0.001$) than those for children with adequate vitamin A levels. Furthermore, VAD is related to low serum iron levels[38] but does not lead to changes in serum ferritin levels; that situation is characteristic of VAD anemia.[41]

In addition, most of the intergroup differences in serum retinol were significant; from highest to lowest as follows: control > FeivAs > FesvAi = FeivAi > FesvAd. Dietary vitamin A content decreased in that order regardless of the dietary iron content. Therefore, under the conditions of this experiment, iron content does not influence serum retinol levels.

Previous research on the effect of vitamin A on iron metabolism has not produced consistent results with regard to the specific effect that vitamin-A-restricted diets have on Hgb concentration. In one study,[6] VAD increased Hgb concentration, but there was no evidence that this change was because of a lack of hemoconcentration. In contrast, another study[2] found mild anemia in both animals and humans when vitamin A was deficient. The current study's results are more consistent with those of still other authors,[23] who found no relationship between vitamin A status and Hgb concentration when iron status is normal. Given these 3 situations, one suggestion is that there is no direct relationship between Hgb concentration and vitamin A unless iron is deficient.

MCV allows for the classification of anemia intro 3 groups based on the morphologic approach: microcytic (below normal), normocytic (within the normal range), and macrocytic (above normal).[11] In comparison to the control group, only the groups that were low in dietary iron had significantly different MCV values; multiple comparisons showed that the groups with sufficient dietary iron (FesvAs, FesvAi, and FesvAd) had significantly higher MCV values than did the groups with ID (FeivAs, FeivAi). In addition, when compared with the reference value of 62.38 ± 1.42 fL, the groups without dietary ID were normocytic, but those with dietary ID were microcytic.[31] In addition, the results showed that only ID (not VAD) influences MCV.

All the groups were within or slightly above the normal range for RBC count, which means that the RBC count can be normal, regardless of the Hgb concentration in the blood, even in ID.

Reticulocyte count is an indicator of erythropoiesis. The current study's results suggested that both iron and vitamin A are necessary for adequate erythropoiesis, as da Cunha and colleagues[15] suggested. Researchers recently have found that vitamin A has a negative effect on hepcidin and an indirect negative effect on erythropoiesis.[16,20] These results are the opposite of the findings of other authors,[37] who suggested that there was no evidence of a relationship between VAD and ineffective erythropoiesis.

Hct is the percentage of RBC (by volume) in the blood. It is determined by multiplying the RBC count by the MCV. Because the RBC counts were similar for all groups and because only the ID groups showed low MCV values, Hct was low (51.3% ± 1.4%) only for the ID groups, as expected. Low Hct values are an indicator of anemia.[21] As with Hgb and MCV, Hct was below the reference range for the iron-deficient groups, again as expected.

MCH is a measure of the average mass of Hgb per RBC, and it is calculated as Hgb × 10 / RBC count.[1,11] Our study's results indicated the presence of hypochromic anemia for the FeivAi group but not for the FeivAs group. Hypochromic anemia occurs when individual cells contain less Hgb than they would have under optimal conditions. Therefore, the results for the iron-insufficient groups showed no direct relationship between Hgb and dietary vitamin A; only the group with insufficiencies

in both vitamin A and iron was hypochromic. Therefore, the results demonstrated vitamin A's effect on the Hgb content of individual cells.

MCHC is an indicator of the average concentration of Hgb in a given volume of packed red blood cells, and it is calculated by dividing Hgb content (in g/100 mL) by Hct (as %). As a fraction, MCHC is spuriously decreased or increased when an accurate measurement of Hgb and hematocrit is disturbed, and increased values are always an artifact because RBC cannot contain more Hgb than normal. Truly increased MCHC usually occurs in hereditary spherocytosis, and reduced MCHC is a reliable representative of functional iron deficiency, even in the absence of anemia.[8,34]

According to our study's results, neither VAD nor ID influences MCHC, but this conclusion is inconsistent with the results for Hgb and Hct, so it is necessary to consider multiple parameters to ensure an adequate assessment of iron's nutritional status. One group of researchers[32] proposed a complete algorithm for diagnosing and classifying anemia by using varying biochemical and erythrocytic parameters, stating that a combination of indicators is necessary for such an assessment.

To assess body weight, we used previously established reference curves[14] that were created to classify the animals into 5 groups according to their nutritional status: malnourished, thin, eutrophic, overweight, and obese (Figure 2) The rats whose diets had any type of nutritional restriction had lower body weights than did those in the control group. At the end of the experiment, we classified all the animals in the experimental groups as eutrophic; in the control group, 4 animals were eutrophic, and the remaining 3 animals were overweight.

Weight gain is a complex process that involves the interaction of many nutrients and metabolic reactions. This study's results are consistent with previous findings,[15] in support of the hypothesis that both iron and vitamin A are required to ensure adequate growth and weight gain. Regarding the classification of nutritional status, the unrestricted use of feeding caused the potential for weight gain, as expected. As one group of researchers[30] suggested, the amount of feed could be reduced by 25% without affecting the animals' physiology; this would reduce their morbidity and increase their life span.

Our current study's results suggested that the lack of either vitamin A or iron can influence animals' growth, regardless of their status as eutrophic or overweight. Because iron is necessary for adequate metabolism and for acquiring energy from foods,[35] it makes sense that iron-insufficient diets reflect inadequate growth. Regarding VAD, researchers have shown that it can affect the function of the mitochondria by causing oxidative damage,[7] which causes further effects in terms of fat metabolism and weight gain. One author[5] suggested that vitamin A influences the methylation patterns of adipogenesis-related genes, thus also potentially explaining the difference in weight gain between the groups with deficient or insufficient vitamin A (FesVAd, FesVAi, FeiVAi) and those with sufficient vitamin A.

Considering the interaction of vitamin A and iron, our results are consistent with the previous evidence regarding the effect of VAD on iron metabolism. For example, some colleagues[23] have suggested that VAD affects the expression of iron regulatory protein 2 and increases ID. In addition, others[15] suggested that VAD affects erythropoiesis and iron metabolism; thus, VAD has an indirect effect on weight gain by regulating iron metabolism.

In conclusion, VAD anemia was not characterized as a distinct clinical entity in 2002.[41] In the 17 y since then, scholars have provided little explanation regarding the mechanisms by which VAD could be involved in the pathogenesis of this kind of anemia. The results of our current study indicate that VAD does not influence all hematologic parameters, because both deficiencies seem to affect only reticulocyte count in young male Wistar rats. Therefore, vitamin A must be considered in the assessment of iron-related nutritional status and in the identification of some types of anemia.

Classification	Body weight (g)
Malnourished	<241.0
Thin	241.0–259.8
Eutrophic	259.8–330.4
Overweight	330.4–347.1
Obese	>347.1

Figure 2. Classification of 9-wk-old male Wistar rats by body weight.[14]

Our current results also suggest that, with regard to body weight, both iron and vitamin A are essential to adequate growth and weight gain; therefore these nutrients must be considered together in nutritional recovery programs. We also remark that, under experimental conditions, Wistar male rats with a regular supply of iron and vitamin A can gain body weight under regular conditions with free-choice feeding.

Finally, formulating a more detailed explanation of the pathogenesis of anemia that is linked to deficiencies in nutrients other than iron (specifically, VAD anemia) might be possible by using analytical techniques such as PCR analysis, Western blotting, and immunohistochemistry. Such techniques might be used to provide additional information about the mechanism through which vitamin A regulates iron metabolism, as well as the cause of nonID anemia at the cellular level.

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