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Copper and Iron Levels in Cow Meat: A Case of the Dietary Intake of the Urban Population in Enugu State, Nigeria

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Abstract
The study assessed the concentration of iron and copper in the muscle and edible offals of Nigerian raised cow and also estimated the dietary intake of these metals through cow meat consumption. The dried meat samples were digested with a 3:2 $HNO_3/HClO_4$ mixture and analysed for the metals with an AAS while the dietary intake was estimated using a one week food frequency questionnaire administered to 755 subjects over a period of three years. The ranges of detectable values ($mgkg^{-1}$) were Cu (2.55-77.42) while Fe (89.17-372.23). Except for 83% of liver samples, the other parts were below the FAO/WHO permissible limit for Cu. Results of the weekly intake of different meat parts in µg/kg body weight /week by the different population studied were in the range; adult men (6.51-134), adult women (6.51-185), pregnant/lactating women (4.38-146), undergraduate students (7.2-184) and school children (10-345) for copper while iron gave: adult men (421-1060), adult women (227-1058), pregnant/lactating women (153-701), undergraduate students (252-1036) and school children (341-1692). The weekly intakes of the two metals were lower than the provisional tolerable weekly intake (PTWI) as stipulated by Joint FAO/WHO Expert Committee on Food Additives (JEFCA). However, higher % values of PTWI were recorded for school children.
Key Words: Copper, Iron, Cow, Dietary intake, Nigeria

1. Introduction

Cattle production is one of the most important forms of agriculture in Nigeria and beef meat is an important part of the human diet. About 99.5% of cattle production are traditionally managed (Federal Ministry of Agriculture 2008). In this method, cattle are free ranged and are predominantly fed on locally grown fodder. Thus, farm animals such as cattle, sheep e.t.c, reared freely on pasture are exposed to metal contamination and could be good indicators of environmental pollution. Trace elements bioaccumulate in tissues of animals by respiration of polluted air and intake of contaminated fodder and water (Tahvonen 1996). The impact of minerals such as copper (Cu) and iron (Fe) on growing cattle is well known. These minerals are needed in sufficient quantities to promote health and to optimize production and reproduction (Ward and Spears 1997).

Copper and iron are among the essential human elements commonly referred to as trace elements or micronutrients. They are termed trace elements (micronutrients) because they are present in animal tissues at concentration of less than 0.005% body weight or for the fact that their requirements in humans and animals are less than 100mg/day (Johnson and Sayberlich 1982; Wolf 1982). These trace elements act as catalysts in a variety of enzyme systems. In this respect, their roles range from weak ionic enzymatic cofactors to highly specific metalloenzymes (Underwood 1977). The trace elements function in the enzymes by direct participation in catalysis, combination with substrate to form a complex upon which the enzyme acts, formation of metalloenzymes, which bind with the substrate, combination with a reaction product and/or maintaining the quaternary structure of the enzymes (Krause and Mahan 1999).

Copper is also a critical functional component of a number of essential enzymes known as coproenzymes including, cytochrome oxidase, Lysyl oxidase, ceruloplasmin, Dopamine-B-monooxygenase, monoamine oxidase and tyrosinase. Copper is also a critical functioning component of the cuproproteins which are involved in energy production, connection tissue formation, iron metabolism, neurotransmitter synthesis and metabolism, functioning and maintenances of myelin, melanin formation, participation as an antioxidant and regulation of gene expression (Lavender 1997; Johnson 1992). In the body, copper shifts between the cuprous (Cu^{+}) and the cupric (Cu^{2+}) states and its ability to accept and donate electrons explains its important role in the oxidation - reduction and in the scavenging of free radical (Linder and Hazegh - Azam 1996).

Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport (Institute of Medicine 2001; Dallman 1986). It is also essential for the regulation of cell growth and differentiation (Bothwell et al. 1979; Andrews 1999). A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity (Institute of Medicine 2001; Haas and Brownlie 2001; Bhaskaram 2001). On the other hand, excess amounts of iron can result in toxicity and even death (Corbett 1995).

The variety of human diet and the frequency of consumption make food intake a potential major environmental influence on health (Kohlmeier 1995). Thus, in other to evaluate the safety and compare the recommended daily intake of human foods, it is necessary to know the amount of these metals taken in the daily

diet. This study was carried out in order to determine the levels of Fe and Cu in the muscles and internal organs of cow and thus estimate their dietary intake from cow meat consumption.

2. Experimental

2.1 Metal analysis

2.1.1. Sample collection and preparation: One hundred and fifty meat samples comprising of 30 each of muscle, liver, kidney, intestine and tripe from 30 cows slaughtered in the abbatoir in Nsukka and Enugu were purchased between August 2007 and November 2008. The cows sampled were between 1-5 years of age and of the White Fulani breed. The samples were dried in an oven at 105^{o}C for six hours, pulverized with a porcelain mortar and kept in acid leached polyethene bags in a dessicator prior to digestion.

2.1.2. Digestion: 2.00 g of each dried meat sample was weighed into a tight fitting polyethylene bottle. The samples were spiked with a solution containing copper and iron and 10 mL 3:2 HNO_3 (65%v/v): $HClO_4$ (70% v/v) were added. The mixture was allowed to digest and then allowed to stand overnight. It was later heated for 3 hours in a water bath adjusted to 70^{o}C with occasional swirling at 30 minutes interval to ensure complete digestion. Finally, the digest was allowed to cool and then transferred into a 20 mL standard flask, rinsing with de-ionized water and later made up to mark with de-ionized water. The solutions were transferred into acid-leached polyethylene bottles and kept at room temperature until analysis with AAS model GBC Avanta ver 2.02, equipped with air-acetylene flame. Unspiked samples were digested by the same procedure while sample blanks were prepared by taking 10 mL of the reagents mixture through the same procedure. On obtaining good recoveries, all samples were prepared by the same procedure.

2.2. Dietary Intake analysis

2.2.1 Study area:

The study was carried out among the people living in the two major urban towns in Enugu State namely Enugu, the state capital and the university town, Nsukka. The socio-demographic data on the subjects shows that about 80% of the subjects were civil servants and about 90% are within middle class and low income earners.

2.2.2 Subjects:

The study involved 755 subjects comprising of adult men (186), adult women (214), pregnant women/lactating women (96), undergraduate students (99) and school children (160). These were categorized in ages (years) as follows: adult men and women 25-55, pregnant/lactating 25-45, undergraduate students 16-25 and school children 6-14.

2.2.3 Method

The daily consumptions of the different parts of meat by the subjects was estimated using a food frequency questionnaire (FFQ) administered personally to the subjects from 2007-2010. One week dietary intakes of the different parts of meat were estimated with the help of the subjects and body weights were recorded. Information on portion size were provided using a two dimensional pictures of cooked meat parts of different sizes ranging from large, moderate, small, and very small (Hankin 1986; Pietinen *et al*. 1988; Posner *et al*. 1992; Williamson *et al*. 2003). The cooked meat parts were previously weighed and photographed with a digital camera and the weights recorded. Data from the FFQ were analysed using SPSS ver 15.0 for windows and dietary intake of the metals per kg body weight was calculated by multiplying the mean weekly consumption of the meat parts by the mean metal concentrations in the meat and dividing with the mean body weight of the different groups studied.

3. Results and Discussion

Triplicate recoveries obtained were in the range: Cu (92-97%) and Fe (98-100%). The concentrations of the metals in the different samples are presented on Tables 1 and 2 respectively. Mean concentrations of Cu in the different parts of meat follow the order: liver > kidney > intestine > tripe > muscle. The high values observed in liver and lowest value in muscle agreed with reports by Johrem *et al*. (1989) in Sweden with liver and muscle concentrations of 39 and 0.87mg/kg respectively, Falandysz (1993) who reported 29 and 1.2 mg/kg in Poland and 44.21 and 1.99mg/kg reported for liver and muscle in cattle from Slovakia (Korenekova *et al.* 2007). According to Benemariya *et al*. (1993), Cu accumulates mainly in the liver. Ruminants have a superior capacity to bind Cu in the liver and have relatively poor Cu excretion. The characteristic of high Cu storage capacity in ruminants means that they are likely to be good bio-monitors of environmental Cu levels and can be used for identification of areas of Cu contamination. The concentrations of Cu in the other meat parts were below the permissible limits of 30mg/kg set by FAO/WHO, but 83% of liver exceeded this limit. The mean concentration of Cu in liver from this study is higher than copper concentration (wet tissue weight) 56.7 mg/kg in livers of Flemish cattle, 8.8 mg/kg in Irish cattle and 22.1 mg/kg in Polish cattle (Zmudzki *et al*. 1991). A comparison of reported values of Cu in muscle, liver and kidney of cattle from various countries as shown in Table 3 shows that the Cu levels in this study were higher than most of the reported values except in few cases. Copper is an essential element, being necessary in various proteins and enzymes. Balance studies on adults suggest an essential daily intake of 1-5 mg (Queensland Health 2002). The absorption and retention rates for copper depend on daily intake. The normal human liver regulates the amount of copper in the body and excretes any excess

through the biliary system. As a consequence of these mechanisms, copper overload is unlikely. In humans with certain rare genetic conditions (copper metabolic disorders) this element can accumulate and cause disease.

Figure 1 shows the mean dietary intake of copper from muscle, liver, kidney, intestine and tripe for the different groups of the subjects. The values were obtained by multiplying the mean copper levels (Table 2) by the mean weekly consumption (Table 4) and dividing with the mean kg body weight (Table 5) for the different groups studied. The trend of mean dietary intake of copper from meat by adult men, adult women and school children is liver> kidney> intestine> tripe> muscle while for pregnant/lactating woman and undergraduate students, the trend is liver> intestine> kidney> tripe> muscle. The consistent higher intakes from liver could be attributed to the very high concentration of copper in the liver. None of the average weekly intake per kg body weight of the different parts of meat studied for the different groups exceeded the PTWI value of 3500µg/kg body weight per week as shown on Figure 1. The highest % mean intake (10%) of PTWI value was recorded in the liver for school children. The low % intakes of PTWI of copper for the different groups show a very low exposure to this metal through cow meat. The dietary intake of copper of liver and kidney in our study was higher than 4270 and 539 µg per person per week reported by MAFF for liver and kidney respectively (MAFF 1998) while 413 µg per person per week reported for muscle in our study was higher than 246 µg per person per week reported for meat (beef, mutton and chicken) in Lahore (Talib 1991). However, the intake for undergraduate students was lower than 2380 µg per person per week reported for female university students in Malaysia (Zawaih and Rosmiza 1995).

Copper deficiency may result in neutroperia (low levels of neutrophils), a condition accompanied by increased susceptibility to infections. Other conditions include osteoporosis in low birth weight infants and young children. Less common features of copper deficiency include loss of pigmentation, neurolological and impaired growth (Uauy 1998). Individuals at risk of copper deficiency include premature babies, individuals with malabsorption syndrome including celiac disease, sprue and short bowel syndrome due to surgical removal of a large portion of the intestine (Uauy 1998).

The trend of mean iron concentration in the meat part studied are liver > tripe > kidney > intestine > muscle. The mean concentrations of Fe in the various meat parts were higher than reported values elsewhere (Table 3). Figure 2 shows the mean dietary intake of iron from muscle, liver, kidney, intestine and tripe for the different groups of the study. The trend of mean dietary intake of iron by adult men and undergraduate students is tripe>liver> intestine> kidney> muscle, for adult women and school children, the trend is tripe> liver> kidney> intestine> muscle while for pregnant/lactating women, the trend is liver> tripe> intestine> kidney> muscle. None of the average weekly intake per kg body weight of the different parts of meat studied for the different groups exceeded the PTWI value of 5600 µg/kg body weight per week as shown on Figure 2. The highest % mean intake (30%) of PTWI was recorded in tripe and liver for school children. The low % intakes of PTWI of iron for the different groups show a very low exposure to the metal. The dietary intake of iron by undergraduate students in our study was lower than 26600 µg per week reported for female undergraduate students in Malaysia (Zawaih and Rosmiza 1995). Most times iron is a deficiency problem, low dietary intake and blood losses can cause iron deficiency. Because so much of the body's iron is in the blood, iron losses are greatest whenever blood is lost. Active bleeding ulcers, menstruation, sand injury result in iron losses. Women are especially prone to iron deficiency during their reproductive years because of repeated blood losses during menstruation (Hoffman, *http://www.drhoffman.com/page.cfm/120*). Pregnancy places iron demands on women as well since iron is needed to support the added blood volume, the growth of the fetus and blood loss during childbirth. Infants and young children receive little iron from their high milk diets, yet extra iron is needed to support their rapid growth. The rapid growth of adolescence, especially for males, and the menstrual losses of teen females demand extra iron that a typical teen diet may not provide.

4. Conclusion

The copper and iron levels in the muscle and internal organs of cow raised in Nigeria are moderate. However, this study reported high levels when compared to data from available literature. The present work has shown that liver contains more of copper and iron than the other parts. Thus, it will serve as a good source of these micro elements to pregnant/lactating women, children and women especially those with heavy menstrual losses.

The dietary intakes of the metals were below the PTWI values. The low intakes indicate a low exposure to these metals from cow meat and this could be attributed to the poor economic status of the subjects. However, higher % intake of PTWI values was recorded for school children for the different meat parts as children consume more food per kilogram of body weight than adults.

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Table 1: Concentrations of Fe in cow meat parts (mg/kg, dry weight) in Nigeria.

Samples	**M**	**L**	**K**	**I**	**T**
1	50.02	241.47	360.20	66.69	632.58
2	96.33	186.73	208.51	86.81	91.11
3	114.76	258.30	219.06	154.14	76.23
4	54.77	152.78	594.55	95.06	759.95
5	58.53	168.04	146.86	103.20	236.99
6	100.82	1697.37	916.47	202.50	673.72
7	135.13	1506.99	492.47	244.23	141.01
8	99.29	454.46	319.90	260.91	671.88
9	72.28	274.91	259.74	44.52	196.76
10	95.41	492.89	364.31	83.49	2408.07
11	68.45	360.82	328.16	93.61	456.35
12	87.01	137.92	199.10	149.83	475.90
13	123.48	112.87	178.90	53.02	193.02
14	95.19	319.20	279.45	77.27	1185.82
15	81.71	314.95	404.73	143.86	207.74
16	77.53	445.64	288.17	167.84	157.94
17	102.95	227.06	240.15	53.09	113.03
18	51.10	245.86	240.00	121.73	145.76
19	151.64	626.95	189.32	78.20	205.10
20	61.54	281.75	185.64	66.12	156.71
21	123.93	193.13	238.82	121.42	83.19
22	75.32	127.24	205.92	172.75	131.58
23	91.56	295.19	274.09	526.61	343.98
24	106.14	448.59	224.10	95.80	158.68
25	94.52	362.33	158.68	75.51	112.84
26	124.12	295.43	212.71	90.16	252.60
27	87.16	243.73	233.14	84.57	107.47
28	55.31	268.21	177.38	111.64	91.86
29	41.99	193.46	240.73	96.57	240.86
30	97.00	232.48	221.67	155.51	98.95
Mean± SD	**89.17±27.27**	**372.23±355.32**	**286.74±154.49**	**129.22±92.32**	**360.26±465.95**

M= muscle, L= liver, K= kidney, I=intestine, T= tripe

Table 2: Concentrations of Cu in cow meat parts (mg/kg, dry weight) in Nigeria.

Samples	**M**	**L**	**K**	**I**	**T**
1	2.00	186.50	14.58	3.70	4.35
2	3.59	97.58	17.50	5.27	3.80
3	3.71	94.54	15.18	9.62	3.41
4	3.55	11.58	10.87	4.45	5.12
5	2.38	113.76	9.87	4.10	3.65
6	3.35	33.53	12.03	4.32	5.94
7	2.35	35.81	13.47	4.82	2.13
8	1.10	57.57	14.37	3.45	4.85
9	2.38	192.29	11.97	4.24	2.82
10	3.33	16.10	14.13	2.84	4.11
11	2.60	98.88	15.43	3.05	3.65
12	1.70	61.28	13.93	2.57	2.45
13	3.40	102.18	10.57	2.06	2.72
14	2.36	158.98	13.60	3.35	4.53
15	1.57	63.57	16.11	4.22	4.59
16	0.92	50.80	11.69	4.52	2.41
17	1.55	51.11	12.20	5.10	2.42
18	1.43	157.55	10.18	3.41	3.11
19	5.26	26.77	10.33	4.05	3.46
20	1.25	34.01	9.61	4.10	3.89
21	4.97	82.09	9.11	4.29	3.39
22	1.27	26.07	12.58	4.69	3.57
23	1.32	132.62	10.13	5.23	2.77
24	2.91	99.36	14.31	4.79	3.11
25	2.67	61.78	9.74	3.37	2.59
26	5.28	36.98	14.15	1.78	3.09
27	1.15	43.57	11.64	3.41	3.21
28	2.21	3.90	8.93	2.91	3.06
29	1.02	77.99	11.76	3.23	4.46
30	4.03	113.98	9.27	4.72	2.98
Mean± SD	**2.55±1.27**	**77.42±51.08**	**12.31±2.32**	**4.06±1.38**	**3.52±0.91**

M= muscle, L= liver, K= kidney, I=intestine, T= tripe

Table 3: Mean concentration ($mgkg^{-1}$) of some heavy metals reported elsewhere.

Countries	Meat parts	Cu	Fe	Reference
Pakistan (Lahore)	Muscle	0.50	-	Talib (1991)
	Muscle	81.51	-	Mariam *et al* (2004)
	Liver	93.24	-	
	Kidney	5.42	-	
Spain	Muscle	1.46	54.4	Miranda *et al* (2005)
	Liver	26.6	96.7	
	Kidney	3.97	108	
Slovak	Muscle	4.433-6.312	49.133-51.800	Korenekova *et al* (2002)
	Liver	31.067-84.098	125.226-146.825	
	Muscle	1.99		Korenekova *et al* (2007)
	Liver	44.21		
Kenya	Muscle	0.037-0.039	-	
	Liver	0.070-4.751	-	
	Kidney	0.076-0.888	-	Oyaro *et al* (2007)
Korea	Muscle	1.73	-	Ji-Hun *et al* (1999)
Lithuanian	Muscle	0.21		Jukna *et al*, (2006)
	Liver	6.91		
	Kidney	3.81		
Nigeria (Enugu)	Muscle	2.55	89.17	This study
	Liver	77.42	372.23	
	Kidney	12.31	286.74	
	Intestine	4.06	129.22	
	Tripe	3.52	360.26	

Table 4: Mean daily consumption (g/day/person) of the different parts of cow meat by different categories of the subjects.

Groups	Meat parts	Mean daily consumption g/day per person ± SD	Mean weekly consumption (g/week per person)
Adult men	**Muscle**	25.54±2.20	178.78
	Liver	17.31±2.86	121.17
	Kidney	11.42±1.61	79.94
	Intestine	32.58±6.10	228.06
	Tripe	29.42±5.27	205.94
***Adult women**	**Muscle**	22.96±2.44	160.72
	Liver	21.47±3.38	150.29
	Kidney	10.77±1.44	75.39
	Intestine	23.50±3.03	164.50
	Tripe	26.42±2.89	184.94
Pregnant/lactating women	**Muscle**	22.56±2.90	157.92
	Liver	24.75±4.87	173.25
	Kidney	12.86±2.68	90.02
	Intestine	40.94±14.39	286.58
	Tripe	22.74±4.38	159.18
Undergraduate students	**Muscle**	26.27±2.93	183.89
	Liver	22.01±4.46	154.07
	Kidney	8.36±1.34	58.52
	Intestine	25.89±5.78	181.23
	Tripe	26.71±5.56	186.97
School children	**Muscle**	19.13±1.83	133.91
	Liver	22.31±3.76	156.17
	Kidney	11.07±2.45	77.49
	Intestine	21.67±4.16	151.69
	Tripe	23.48±4.55	164.36

*excludes pregnant/lactating women

Table 5: Average weight (kg) of the different groups of the subjects

Groups	N	Range(kg)	Mean (kg)	SD
Adult men	186	50-103	70	10.36
Adult women	214	45-91	63	10.80
Pregnant/lactating women	96	70-125	92	12.19
Undergraduate students	99	45-101	65	10.83
School children	160	21-57	35	5.91

SD- standard deviation

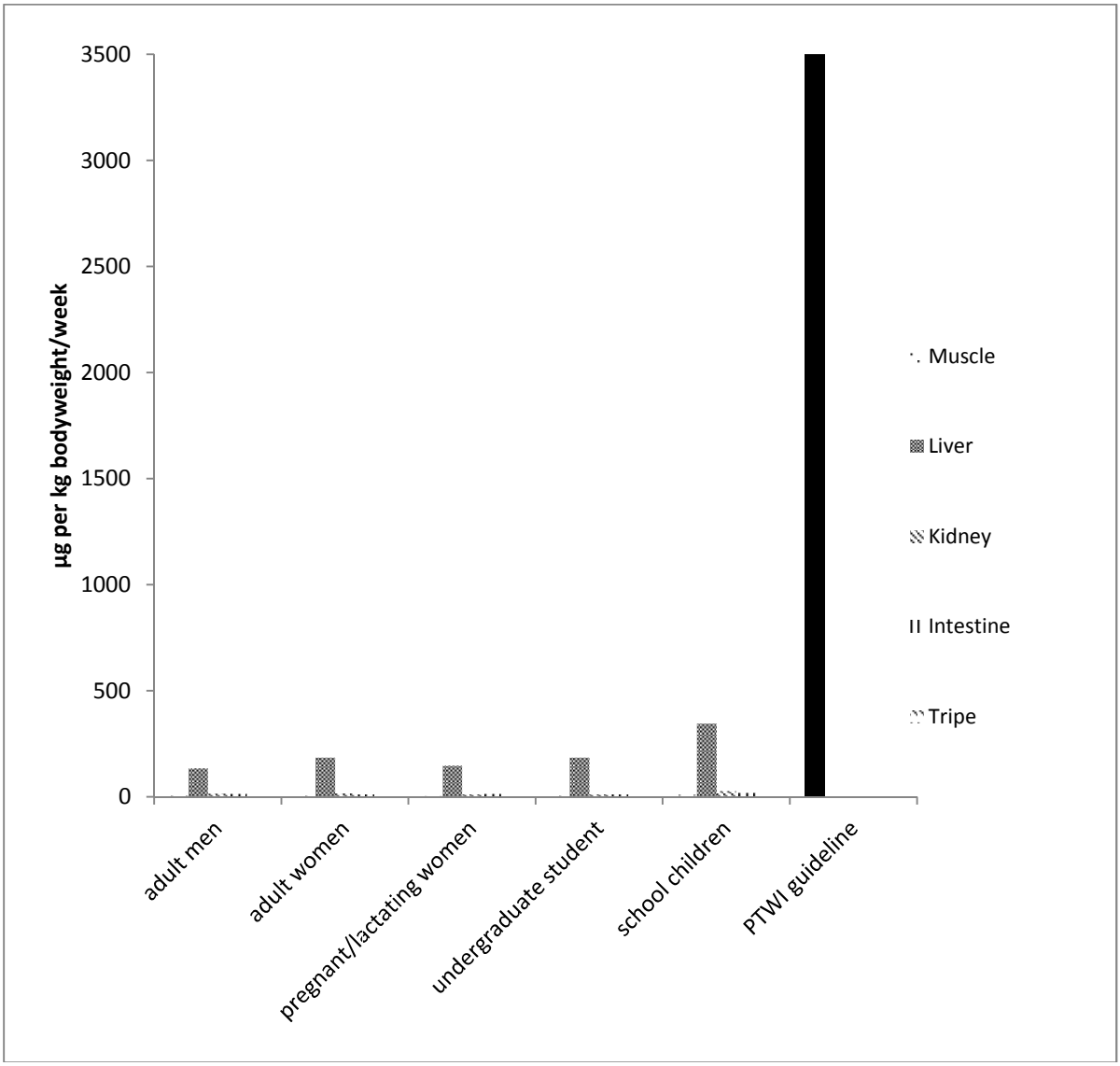

Figure 1: Mean dietary intake of copper by the different categories of the subjects.

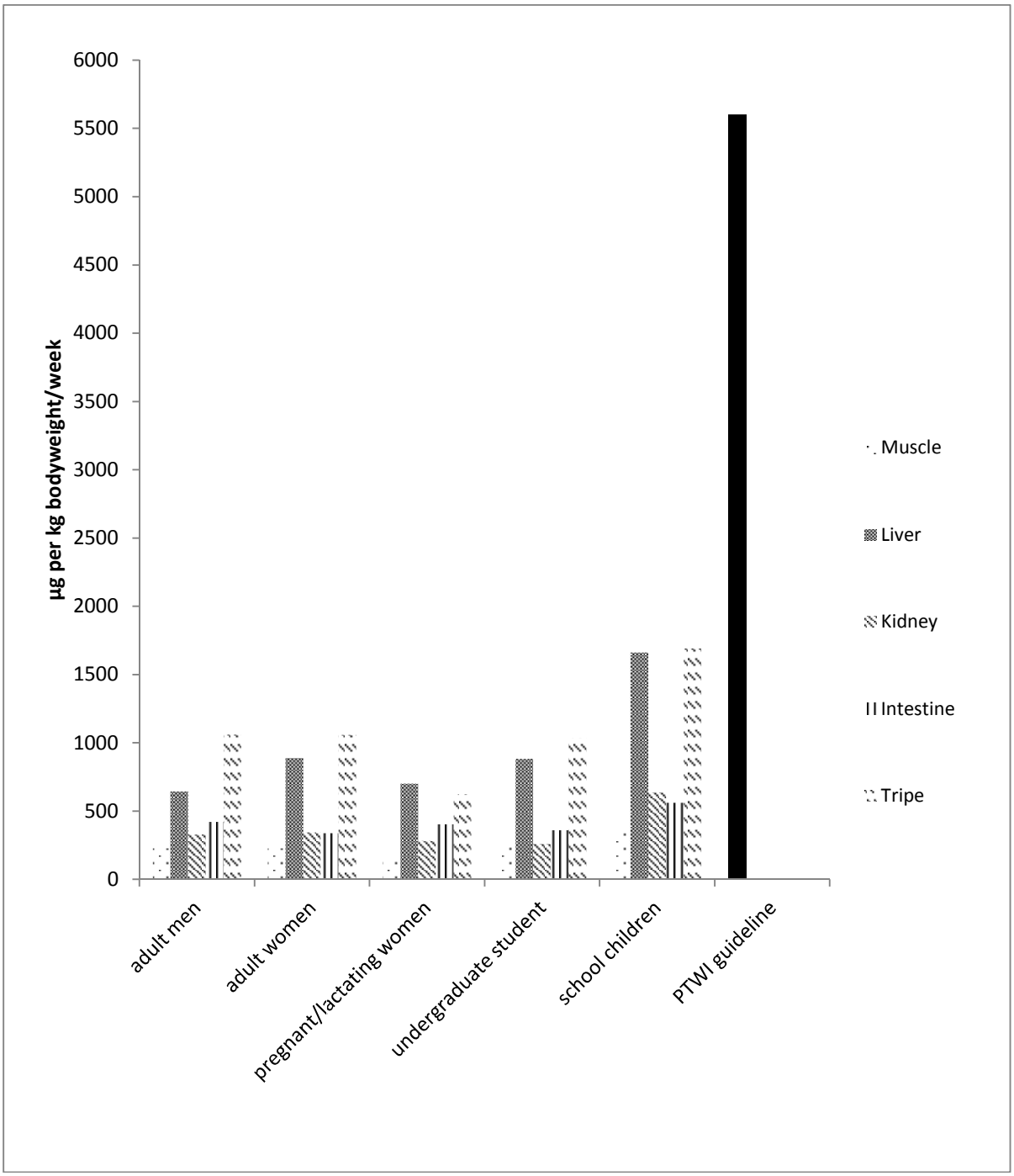

Figure 2: Mean dietary intake of iron by the different categories of the subjects