Chapter 4

Findings and Result

4.1 Percentage yield of rice bran extract

Each portion of 120 g rice bran was extract by warm water at 70 and 90 ˚C. The extract at 70˚C was also done in the presence of enzyme mixture. The yield was expressed as g dry weight and percentage (Table 4.1).

Table 4.1

The percentage yield of rice bran by water extract at various temperature

Extraction temperature (˚C)	Time (min)	Raw material (g)	Percentage yield (%) (Mean±S.E.M)
70	60	120	11.98±1.12
70†	60	120	12.79±1.31
90	60	120	13.33±2.01

† The water extraction was done in the presence of enzyme mixture.

Values are expressed as mean $\pm$ S.E.M., n = 5

There were no significant differences at $p<0.05$.

4.2 Determination of antioxidant activity

The result showed EC_{50} of two kinds rice bran extract, the water extraction at 90˚C and the water plus enzyme mixture at 70˚C, in comparison to BHT and γ-Oryzanol (Table 4.2 and Figure 4.1).

Table 4.2

EC_{50} value (mg/ml) of DPPH free radical-scavenging activity of rice bran extract expressed as mean±SEM. of triplicate assay.

Sample	EC_{50}, mg/ml
BHT	0.012 ± 0.09^{a}
γ-oryzanol	0.029 ± 0.37^{b}
RBE 70 ˚C†	0.155 ± 1.53^{c}
RBE 90˚C	0.331 ± 9.29^{d}

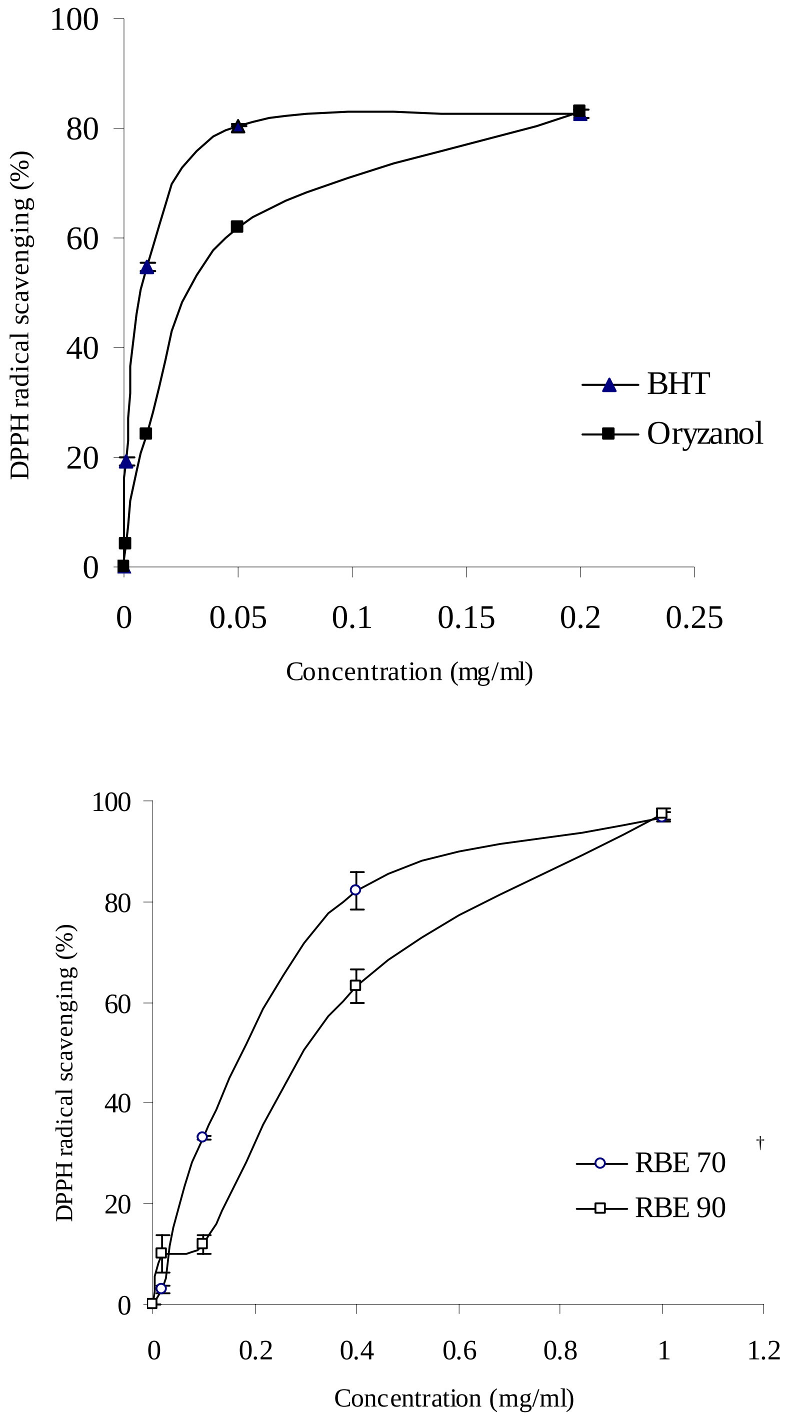
† The water extraction was done in the presence of enzyme mixture.

Results represent means ± S.E.M. (n=3).

^{a,b,c,d} Value in a row not sharing the common superscript letter are significantly difference, $p<0.05$

Figure 4.1

DPPH radical scavenging (%) of BHT, γ-oryzanol and rice bran extract expressed as mean±SEM. of triplicate assay



4.3 Qualitative assessment for carbohydrate and protein of rice bran extract

The position sign indicated the present of biochemical property in accordance to the test. Table 4.3 showed biochemical property of rice bran extracted by water at 70˚C the presence of enzyme mixture. The positive results were also shown in Table 4.2 and Figures 4.2-4.3

Table 4.3

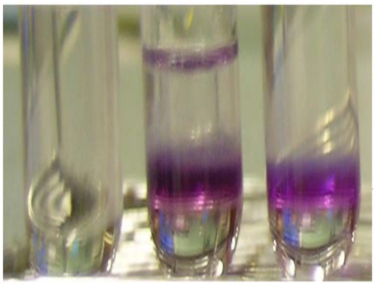
Characteristic of rice bran extract and testing qualitative for carbohydrate and protein

Physical appearance	The powder is brown and fragrant, 	
Qualitative testing for carbohydrate	**Property**	**Results**
-Molisch test	General carbohydrate	+
-Benedict test	Reducing sugar	-
-Barfoed test	Monosaccharide structure	-
-Seliwanoff test	Ketose structure	+
-Bial test	Pentose structure	-
-Iodine test	Polysaccharide structure	+
Qualitative testing for Protein		
-Ninhydrin test	Amino group	-
-Biuret test	Peptide bond	+
-Xanthoproteic test	Aromatic side chain	+
-Hopkins Cole test	Indole ring side chain	+
Millon’s test	Phenolic side chain	+
Sakaguchi test	Guanidine side chain	+

Figure 4.2

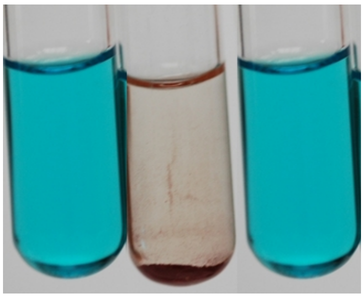
Qualitative testing for carbohydrate

Negative Positive SRB



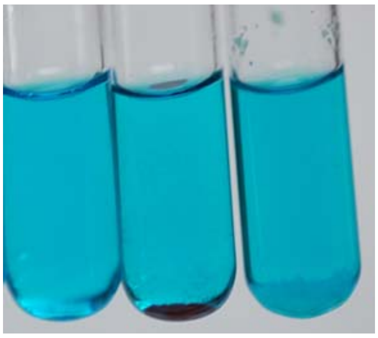
Molisch test

Negative Positive SRB



Benedict test

Negative Positive SRB



Barfoed test

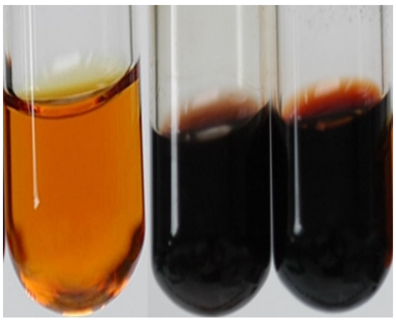
Negative Positive SRB

Seliwanoff test

Negative Positive SRB

Bial test

Negative Positive SRB

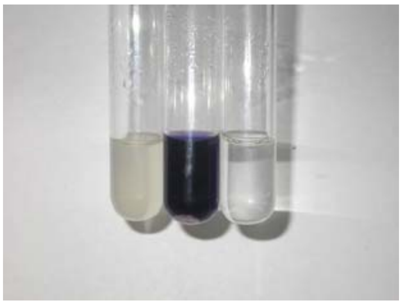


Iodine test

Figures 4.3

Qualitative testing for protein

Negative Positive SRB



Ninhtdrin test

Negative Positive SRB

Biuret test

Negative Positive SRB

Xanthoproteic test

Negative Positive SRB

Hopkins Cole test

Negative Positive SRB

Millon test

Negative Positive SRB

Sakaguchi test

4.4 Effects of rice bran extract on body weight and food intake

Body weight and energy intake in all groups were shown in table 4.4-4.5. The body weight of the group receiving metformin and RBE at 22.05, 220.5 body weight gain tended to be lesser than that of the high-fat diet group. However, the decrease was not significant when compared with the control group. RBE at dose 2205 mg/kg was able to significantly hamper the increase body weight gain when compared with the high-fat diet group (125.98±7.32 vs 160.72±10.03).

The energy intake in all groups those receiving high-fat diet was significantly difference from the control group, even though they were also receiving metformin or rice bran extract

4.5 Effects of rice bran extract on organ weight

Organ weights for in all groups were shown in table 4.6-4.7. Interestingly, abdominal fat were the only tissue that its weight was significant difference between groups. Abdominal fat from rats in the group that fed high-fat diet weighed significantly more than those from rats in the control group. The trend of increasing weight of there abdominal fat were hampered when rats also received rice bran extract. The hampering effect was so effective when rats received the highest dose of rice bran extract that their fat weights were equal to those of the control and metformin group.

4.6 Effects of rice bran extract on glucose homeostasis parameters

The parameters for glucose homeostasis were shown in table 4.8-4.9. Fasting blood glucose levels were higher than the control group when rats were fed with high-fat diet. There was no effect of rice bran extract on fasting blood glucose levels no matter how high the rice bran extract were co-feeding. While metformin was able to hamper the increasing blood glucose level.

The difference result were found regarding to area under the curve of glucose (AUC-G). The rice bran extract dose over or equal to 220.5 mg/kg effectively hampered the increasing AUC-G when rats were fed with high-fat diet.

Although serum insulin level and HOMA-IR were not significant difference among the groups, rice bran extract trended to exert an effect on HOMA-β. The rice bran extract dose over or equal to 220.5 mg/kg was able to increase reducing HOMA- β.

4.7 Effects of rice bran extract on serum lipid levels

Serum lipid levels were shown in Table 4.10-4.11. The rice bran extract dose over or equal to 220.5 mg/kg was as efficient as metfromin in hampering the increasing serum triglyceride level when rats were fed with high-fat diet. Rice bran extract and metformin had no effect on other plasma lipids parameter, except that metformin gave a negative effect (decrease) on HDL-C. Regarding to the high-cholesterol content in the diet, either total blood cholesterol or cholesterol per HDL-C ratio were not increased. There was no statistic difference between cholesterol levels in rats fed with high-fat diet and those fed with standard chow.

4.8 The correlation data

Regardless of treatment, the correlations among parameters were analyzed. The data was shown in table 4.12 and in Figure 4.4-4.5. Pearson correlation between each parameter against weight of abdominal fat pads. Regarding to the type of parameters significant cross correlation between the glucose homeostasis and body lipid found were the negative correlation between HOMA-β and abdominal fat weight and the positive correlation between FBG and abdominal fat weight. While significant positive correlation between serum triglyceride, LDL-cholesterol and fasting blood glucose.

Table 4.4

Effects of high-fat diet on body weight and energy intake

	Control diet	High-fat diet
Body weight (g)		
Before	203.13±4.11[a]	200.00±6.12[a]
After	348.13±8.26[a]	360.71±9.92[a]
Body weight gain	145.24±5.05[a]	160.72±10.03[a]
Energy intake (Kcal)		
Before	64.50±1.55 [a]	65.00±1.08[a]
After	66.76±1.10 [a]	98.61±2.94[b]

Values are expressed as mean ± S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.5

Effects of rice bran extract on body weight and energy intake in high-fat feeding rats

Parameters	High-fat diet	High-fat diet and Metformin 9.55 mg/kg	Rice bran extract on High-fat diet		
			22.05 mg/kg	220.5 mg/kg	2205 mg/kg
Body weight (g)					
Before	200.00±6.12[a]	205.00±2.11[a]	205.63±4.76[a]	195.63±2.20[a]	195.00±7.07[a]
After	360.71±9.92[a]	350.06±3.53[a]	364.46±5.13[a]	351.34±5.47[a]	320.98±9.25[a]
Body weight gain	160.72±10.03[a]	149.33±3.04[a]	158.84±4.16[a]	155.72±5.47[a]	125.98±7.32 [b]
Energy intake					
Before	65.00±1.08[a]	65.42±0.43[a]	66.00±1.08[a]	65.50±0.96[a]	66.25±0.85[a]
After	98.61±2.94[a]	99.40±3.30[a]	99.11±4.32[a]	99.26±4.35[a]	91.28±4.68[a]

Values are expressed as mean ± S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.6

Effect of high-fat diet on organ weights

Organ	Organ weight (g)	
	Control diet	High-fat diet
Brain	1.79±0.04 [a]	1.73±0.35 [a]
Heart	1.88±0.12 [a]	1.64±0.38 [a]
Liver	15.75±0.65 [a]	16.69±0.58 [a]
Pancreas	2.00±0.17 [a]	1.69±0.11 [a]
Intestine	22.07±3.09 [a]	21.79±1.25 [a]
Adrenal	0.08±0.01 [a]	0.08±0.01 [a]
Kidney	1.63±0.06 [a]	1.51±0.04 [a]
Abdominal fat	6.71±0.67 [a]	13.95±0.44 [b]
Thymus	0.53±0.06 [a]	0.85±0.13 [a]

Values are expressed as mean $\pm$ S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.7

Effect of rice bran extract on organ weights in high-fat feeding rats

Organ	High-fat diet	High-fat diet and Metformin 9.55 mg/kg	Rice bran extract on High-fat diet		
			22.05 mg/kg	220.5 mg/kg	2205 mg/kg
Brain	1.73±0.35[a]	1.78±0.03[a]	1.81±0.02[a]	1.73±0.04[a]	1.79±0.02[a]
Heart	1.64±0.38[a]	2.32±0.11[a]	2.28±0.08[a]	2.14±0.05[a]	2.10±0.10[a]
Liver	16.69±0.58[a]	16.17±0.67[a]	16.39±0.79[a]	15.09±0.77[a]	14.73±0.80[a]
Pancreas	1.69±0.11[a]	1.94±0.21[a]	1.79±0.08[a]	1.50±0.13[a]	1.64±0.13[a]
Intestine	21.79±1.25[a]	20.98±0.92[a]	20.99±1.38[a]	19.22±0.85[a]	19.08±0.74[a]
Adrenal	0.08±0.01[a]	0.16±0.08[a]	0.07±0.01[a]	0.07±0.004[a]	0.08±0.004[a]
Kidney	1.51±0.04[a]	1.57±0.05[a]	1.54±0.03[a]	1.47±0.02[a]	1.46±0.05[a]
Abdominal fat	13.95±0.44[a]	6.47±0.42[a]	13.05±0.88[b]	11.24±0.64[b]	8.99±0.72[b]
Thymus	0.85±0.13[a]	0.68±0.03[a]	0.64±0.02[a]	0.63±0.04[a]	0.57±0.04[a]

Values are expressed as mean ± S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.8

Effects of high-fat diet on glucose homeostasis parameters in blood

Parameters	Control diet	High-fat diet
Fasting blood glucose (mmol/L)	5.88±0.16[a]	7.15±0.24[b]
Fasting insulin (mU/ml)	29.11±5.57[a]	30.12±5.45[a]
AUC-G (mg/dL.min)	2248.00±241.59[a]	2787.75±472.54[a]
HOMA-IR	7.44±1.1.67[a]	9.89±1.85[a]
HOMA-β	265.53±63.22[a]	156.17±26.91[a]

Values are expressed as mean ± S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.9

Effects of rice bran extract on glucose homeostasis parameters in high-fat feeding rats

Parameters	High-fat diet	High-fat diet and Metformin 9.55 mg/kg	Rice bran extract on High-fat diet		
			22.5 mg/kg	220.5mg/kg	2205 mg/kg
Fasting blood glucose (mmol/L)	7.15±0.24[a]	6.39±0.39[a]	7.06±0.20[a]	7.07±0.19[a]	7.12±0.25[a]
Fasting insulin (mU/ml)	30.12±5.45[a]	45.45±6.12[a]	28.76±6.71[a]	32.48±6.40[a]	42.28±10.17[a]
AUC-G (mg/dL.min)	2787.75±472.54[a]	2288.125±356.94[a]	1973.56±642.28[a]	1566.85±347.35[b]	1244.83±189.62[b]
HOMA-IR	9.89±1.85[a]	12.76±1.58[a]	8.94±2.15[a]	10.09±1.95[a]	13.58±3.34[a]
HOMA-β	156.17±26.91[a]	329.27±56.33[b]	167.07±37.87[a]	191.95±44.51[a]	235.52±59.08[a]

Values are expressed as mean ± S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.10

Effects of high-fat diet on plasma lipid level

Plasma lipid	Control diet	High-fat diet
Triglyceride (mg/dl)	47.13±3.58[a]	66.13±6.06[b]
Total cholesterol (mg/dl)	65.12±3.19[a]	64.75±2.08[a]
LDL-C (mg/dl)	12.25±1.01[a]	15.00±0.68[b]
HDL-C (mg/dl)	23.00±0.80[a]	20.25±0.56[b]
Total cholesterol/HDL-C	2.83±0.08[a]	3.20±0.25[a]

Values are expressed as mean $\pm$ S.E.M., n = 8

[a,b] Values in a row not sharing the common superscript letter are significantly different, $p<0.05$

Table 4.11

Effects of rice bran extract on plasma lipid level in high-fat feeding rats

Plasma lipid	High-fat diet	High-fat diet and Metformin 9.55 mg/kg	Rice bran extract on High-fat diet		
			22.5 mg/kg	220.5mg/kg	2205 mg/kg
Triglyceride (mg/dl)	66.13±6.06[a]	57.88±6.22[a]	66.13±4.82[a]	62.88±3.29[a]	58.75±9.79[a]
Total cholesterol (mg/dl)	64.75±2.08[a]	58.63±4.97[a]	64.50±4.09[a]	64.75±4.70[a]	63.25±3.23[a]
LDL-C (mg/dl)	15.00±0.68[a]	12.13±1.44[a]	15.00±1.25[a]	15.63±1.19[a]	15.25±1.53[a]
HDL-C (mg/dl)	20.25±0.56[a]	19.13±0.76[a]	20.25±1.22[a]	20.50±1.18[a]	22.25±1.62[a]
Total cholesterol/HDL-C	3.20±0.25[a]	3.04±0.25[a]	3.19±0.25[a]	3.14±0.20[a]	2.89±0.14[a]

Values are expressed as mean ± S.E.M., n = 5

[a] Values in a row sharing a common superscript are not significantly different , $p<0.05$

Table 4.12

Pearson correlation among parameters were analyzed

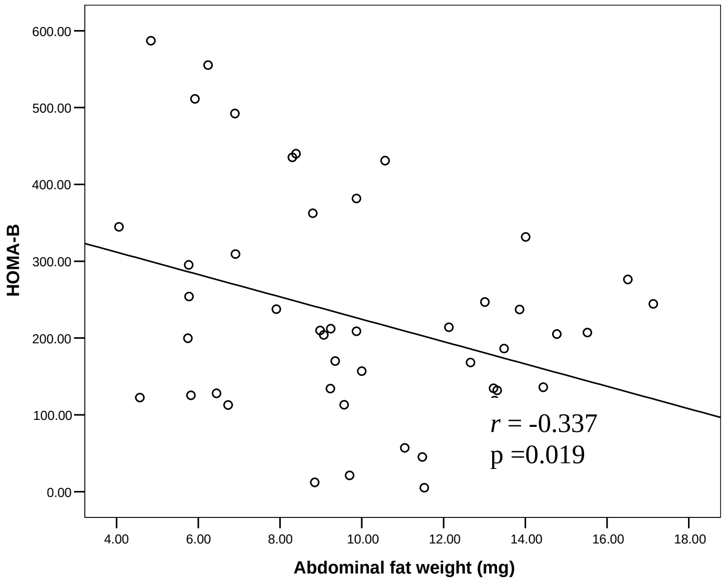
Parameter	Pearson Correlation								
	FBG	HOMA-IR	HOMA-β	Insulin	Triglyceride	Total cholesterol	LDL-C	HDL-C	Abdominal fat pad
AUC_G (mg/dl.min) p-value	-0.160 0.279	0.130 0.380	0.210 0.152	0.161 0.274	-0.048 0.748	-0.048 0.746	-0.092 0.536	-0.073 0.622	0.136 0.358
FBG (mmol/L) p-value	-	0.180 0.222	-0.397** 0.005	-0.007 0.961	0.174 0.238	-0.049 0.742	0.293* 0.043	-0.162 0.270	0.533** 0.000
HOMA-IR p-value	-	-	0.774** 0.000	0.976** 0.000	0.150 0.310	-0.035 0.816	0.018 0.902	0.053 0.722	0.012 0.935
HOMA-β p-value	-	-	-	0.891** 0.000	0.041 0.784	-0.73 0.623	-0.186 0.206	0.140 0.722	-0.337* 0.019
Insulin (mU/ml) p-value	-	-	-	-	0.129 0.383	-0.055 0.709	-0.052 0.725	0.074 0.616	-0.105 0.478
Triglyceride p-value	-	-	-	-	-	0.241 0.098	0.248 0.089	0.347* 0.016	0.329* 0.022
Total cholesterol (md/dl) p-value	-	-	-	-	-	-	0.580** 0.000	0.607** 0.000	0.143 0.333
LDL-C p-value	-	-	-	-	-	-	-	0.500** 0.000	0.326* 0.024
HDL-C p-value	-	-	-	-	-	-	-	-	-0.600 0.684

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Figure 4.4

Correlation between HOMA-β and fasting blood glucose with abdominal fat pads weight



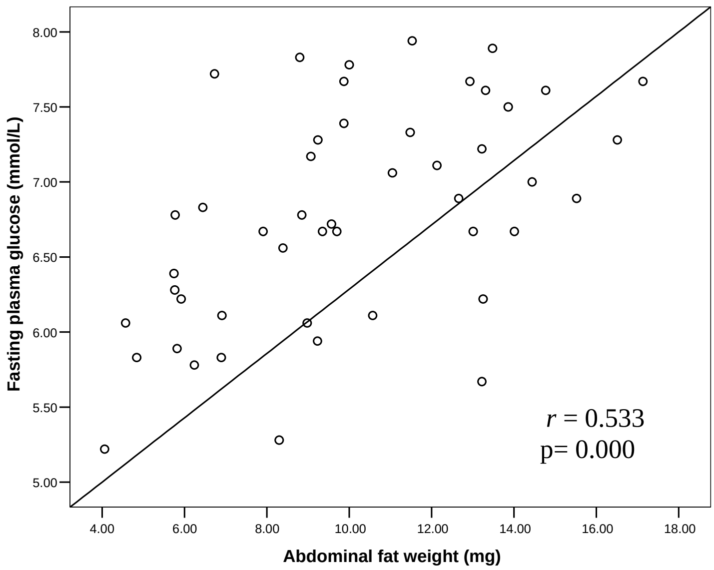


Figure 4.5

Correlation between triglyceride and LDL-cholesterol with abdominal fat pads weight